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# REPORT ON THE CALCAREOUS SPONGES OBTAINED BY THE SURVEY OF THE CONTINENTAL SHELF BORDERING ON JAPAN.

By

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(With Plate I and 4 text-figures)

(Received October 20, 1932)

The calcareous sponges dealt with in the present report were secured by the survey of the continental shelf bordering on Japan. The survey was undertaken by the Imperial Fisheries Institute in Tokyo, and was carried out during the years from 1922 to 1930. The number of stations at each of which the dredging was tried, is very great, reaching 658, and they are widely distributed among the seas surrounding Hondo, Shikoku, and Kiushiu.

The specimens of calcareous sponges which have thus far been obtained, and which were submitted for examination and report are thirteen in all. They represent eight species belonging to five genera and three families. Of these eight species, four are identical with those previously known, while the remaining four are here described for the first time.

In view of the richness of the Calcareous fauna in Japanese waters, it is a matter for inquiry why the species obtained by this survey are so surprisingly few.

The following is the list of the species.

Family Homocoelidae DENDY and ROW.

- 1) *Leucosolenia canariensis* MICHLUCHO-MACLAY.
- 2) *Leucosolenia soyo*, n. sp.

Family Heteropiidae DENDY.

- 3) *Grantessa intusarticulata* CARTER.
- 4) *Amphiute ijimai* HÔZAWA.

Family Grantiidae DENDY.

- 5) *Grantia glabra*, n. sp.
- 6) *Grantia kajiensis*, n. sp.
- 7) *Leucandra dura* HÔZAWA.
- 9) *Leucandra yuriagensis*, n. sp.



I shall next give a list of the stations, showing their position, depth, and the specimens obtained at each.

- Station 61. Lat. N.  $40^{\circ} 3' 24''$ ; long. E.  $142^{\circ} 11' 33''$ ;  
Off Fudai, Rikuchiu;  
depth 170 m. .... *Leucosolenia soyo*, n. sp. (Spec. No. P. 193)
- Station 64. Lat. N.  $40^{\circ} 14' 50''$ ; long. E.  $142^{\circ} 5' 10''$ ;  
Off Kuji, Rikuchiu;  
depth 150 m. .... { *Leucosolenia canariensis* (Spec. No. P. 50)  
                                  *Grantia kujiensis*, n. sp. (Spec. No. P. 52)
- Station 66. Lat. N.  $40^{\circ} 28' 40''$ ; long. E.  $142^{\circ} 1' 51''$ ;  
Off Hachinohe, Mutsu;  
depth 165 m. .... *Leucosolenia canariensis* (Spec. No. P. 32)
- Station 102. Lat. N.  $38^{\circ} 6' 30''$ ; long. E.  $141^{\circ} 11'$ ;  
Off Yuriage, Rikuzen;  
depth 37 m. .... *Leucandra yuriagensis*, n. sp. (Spec. No. P. 107)
- Station 122. Lat. N.  $36^{\circ} 54' 30''$ ; long. E.  $141^{\circ} 17'$ ;  
Off Shioyazaki, Iwaki;  
depth 161 m. .... *Grantia glabra*, n. sp. (Spec. Nos. P. 78, 192)
- Station 271. Lat. N.  $34^{\circ} 58' 3''$ ; long. E.  $139^{\circ} 35' 20''$ ;  
Off Suno-saki, Awa;  
depth 82 m. .... *Grantessa intusarticulata* (Spec. No. P. 184)
- Station 278. Lat. N.  $34^{\circ} 42' 50''$ ; long. E.  $138^{\circ} 30' 30''$ ;  
Seno-umi, Suruga Bay;  
depth 79 m. .... *Amphiute ijimai* (Spec. No. P. 189)
- Station 376. Lat. N.  $34^{\circ} 33' 35''$ ; long. E.  $138^{\circ} 7' 45''$ ;  
Off Omae-zaki, Tôtômi;  
depth 64 m. .... *Leucandra dura* (Spec. No. P. 190)
- Station 600. Lat. N.  $38^{\circ} 33' 37''$ ; long. E.  $138^{\circ} 21' 50''$ ;  
Off Hajiki-zaki, Sado;  
depth 168 m. .... *Leucosolenia soyo*, n. sp. (Spec. Nos. P. 186, 188)
- Station 630. Lat. N.  $39^{\circ} 52' 45''$ ; long. E.  $139^{\circ} 34'$ ;  
Off Tsuchizaki, Ugo.  
depth 150 m. .... *Grantia glabra*, n. sp. (Spec. No. P. 191)

## Class and Order CALCAREA.

### Family Homocoelidae DENDY and ROW.

#### Genus LEUCOSOLENIA BOWERBANK.

#### 1. *Leucosolenia canariensis* (MICHLUCHO-MACLAY)

(Pl. I, fig. 1)

*Nardoa canariensis*, MICHLUCHO-MACLAY, 1868, p. 230.

*Nardoa sulphurea*, MICHLUCHO-MACLAY, 1868, p. 230.

*Nardoa rubra* MICHLUCHO-MACLAY, 1868, p. 230.

*Torroma canariense*, HAECKEL, 1870, p. 244.

*Torroma rubrum*, HAECKEL, 1870, p. 245.

*Ascartis canariensis*, HAECKEL, 1872, p. 52, Pl. 9, figs. 1-3; Pl. 10, figs. 1, a-c.

*Ascartis compacta*, SCHUFFNER, 1877, p. 404, Pl. 25 fig. 9.

*Leucosolenia nansenii*, BREITFUSS, 1896, p. 427; 1898, p. 106, Pl. 12, figs. 1-9,

*Leucosolenia canariensis*, LAKSCHEWITSCH, 1886, p. 300. Pl. 7, fig. 1; THACKER, 1908, p. 762, Pl. 40, fig. 3, text-figs. 157-160; DENDY and ROW, 1913, p. 724; HÖZAWA, 1918, p. 528.

There are in the collection two specimens of this sponge. The first (Spec. No. P. 50; Pl. I, fig. 1) was secured at Station 62 (Lat. N.  $40^{\circ} 14' 50''$ , long. E.  $142^{\circ} 05' 10''$ ) at a depth of 150 m.

The sponge forms an irregularly shaped massive colony composed of Ascon-tubes branching and anastomosing in a very complex manner. It grows vertically upwards, being attached to the substratum by a small number of processes at the base. The colony is more or less flattened from side to side, the surface even showing no irregular ridges and depressions at all, and, moreover, many very small conical papillae formed by the union of Ascon-tubes each of which is without any distinct osculum at its summit are scattered over it. The height of the specimen is about 70 mm., the greatest breadth about 30 mm., and the greatest thickness about 18 mm. The pseudoderm, which is formed by the outer Ascon-tubes is perforated by numerous pseudopores; they are irregularly rounded or oval in shape, and measure about 1 mm. in maximum diameter.

The second specimen (Spec. No. P. 32) which was obtained at Station 66 (Lat. N.  $40^{\circ} 28' 40''$ , long. E.  $142^{\circ} 01' 51''$ ) from a depth of 165 m. is much smaller than the first. It is irregularly shaped, measures about 23 mm. in height, about 17 mm. in the greatest breadth, and about 10 mm. in the greatest thickness. The pseudopores are rather wide compared with those of the first specimen, and attain 2 mm. in maximum diameter.

The texture is fairly rigid in the outer part of the colony but is rather delicate in the interior. The colour turns greyish-white in alcohol.

Structure.—The canal system of this species seems to agree well with that proposed as type B by DENDY<sup>1)</sup>. But, as stated above, the absence of true oscula leading directly into a space lined by collared cells and formed by the union of Ascon-tubes indicates a lipostomous condition.

The skeleton of the walls of the Ascon-tubes is mainly composed of triradiates arranged in several but not many layers in an irregular manner, and there may be added a small number of quadriradiates with their apical rays projecting into the cavity of the Ascon-tubes. The spicules

<sup>1)</sup> DENDY, 1891, pp. 27, 28.

which occur in the wall of the outer Ascon-tubes are, more or less, stouter and are more closely set than in that of those in the interior of the colony.

Spicules.—Triradiates regular with rays rather slender, straight, usually rather bluntly-pointed,  $100-130\ \mu$  long,  $8-12\ \mu$  thick at base.

Quadriradiates similar to triradiates except in the presence of the apical ray. The apical ray which projects at right angles from the centre of the facial rays is nearly straight in the basal parts but rather curved in the apical, terminating in a very fine point. The apical ray is slightly shorter and much narrower than the facial rays,  $80-100\ \mu$  long about  $6\ \mu$  thick at base.

*Localities*.—Canary Islands (MICHLUCHO-MACLAY); Cape Verde Islands (THACKER); Mauritius (SCHUFFNER); Minorca (LAKSCHEWITSCH); Spitzbergen, Arctic Ocean (BREITFUSS); off the north point of Capper Island, Commander Islands (HÔZAWA).

*Remarks*.—This species was first described by MICHLUCHO-MACLAY as having been found in 1868 on the Coast of Lanzerote, Canary Islands. Since that time it has been reported by several investigators such as HAECKEL (1870, 1872), SCHUFFNER (1877), BREITFUSS (1896, 1898), LAKSCHEWITSCH (1886), DENDY and ROW (1913), etc, the specimens being obtained from various parts of the world. In 1908 THACKER gave a full account discussing the synonymy and the affinities of this species.

In 1918 I reported this species as found in the Commanders Islands in Kamchatka, and I am now able to report the same species as having been found in Japanese waters.

Judging by the facts above mentioned, the present species seems to be widely distributed all over the world, and may thus be considered to be cosmopolitan.

## 2. *Leucosolenia soyo*, n. sp.

(Pl. I, fig. 2; text-fig. 1)

Of this species there exist three specimens in the collection.

The first specimen (Spec. No. P. 188; Pl. I, fig. 2) was obtained at Station 600 (Lat. N.  $38^{\circ} 33' 37''$ , long. E.  $138^{\circ} 21' 50''$ ) at a depth of 168 m.

The sponge forms irregularly-shaped lobose masses, conspicuously flattened from side to side, and attached by root-like processes to foreign objects. Judging by its general appearance, as well as by the existence of two pseudoscula, it seems to have consisted originally of two colonies

fused at two points in the basal parts. The greatest breadth of the entire specimen is about 30 mm., and the greatest height about 20 mm. while the thickness is about 3 mm. The pseudopores are thickly and fairly evenly distributed in the pseudoderm, varying in size up to about 0.3 mm. in diameter, and mostly of oval shape.

The second specimen (Spec. No. P. 186), which came from the same station as the first, is represented by only a fragment measuring 15 mm. long and about 6 mm. broad.

The third specimen (Spec. No. P. 193) was obtained at Station 61 (Lat. N.  $40^{\circ} 03' 24''$ , long E.  $142^{\circ} 11' 33''$ ) at a depth of 170 m.

It is irregularly ovoid in shape, and is more or less laterally compressed. The surface is not even, showing ridges and depressions. The lower parts which seem to be attached to the substratum are torn off in this case. The specimen is about 15 mm. long and about 13 mm. broad, while its greatest thickness is about 5 mm.

A single pseudosculum which opens at the top of the sponge is very irregularly formed, and is surrounded by a thin membranous margin. It measures about 3 mm. in maximum diameter.

The pseudoderm, which covers the general surface of the sponge, is perforated by numerous thickly distributed pseudopores of oval or circular shape with a diameter of 0.15–0.4 mm. The following description is based on the first specimen, which is taken as the type.

Structure.—The canal system belongs to DENDY's type D<sup>1)</sup>, though it may not be quite typical.

The pseudogaster opening above by the pseudosculum is very capacious spreading into the interior of the entire specimen.

The membrane which lines the pseudogaster is penetrated by the exhalant openings of the Ascon-tubes, each of these openings leading either into the gastral cavity of a single Ascon-tube, or into two or three of these tubes.

The pseudopores distributed on the pseudoderm lead into interspaces between the Ascon-tubes. These interspaces are wide and nearly straight, and continue right up to the lining membrane of the pseudogaster almost without diminishing in size.

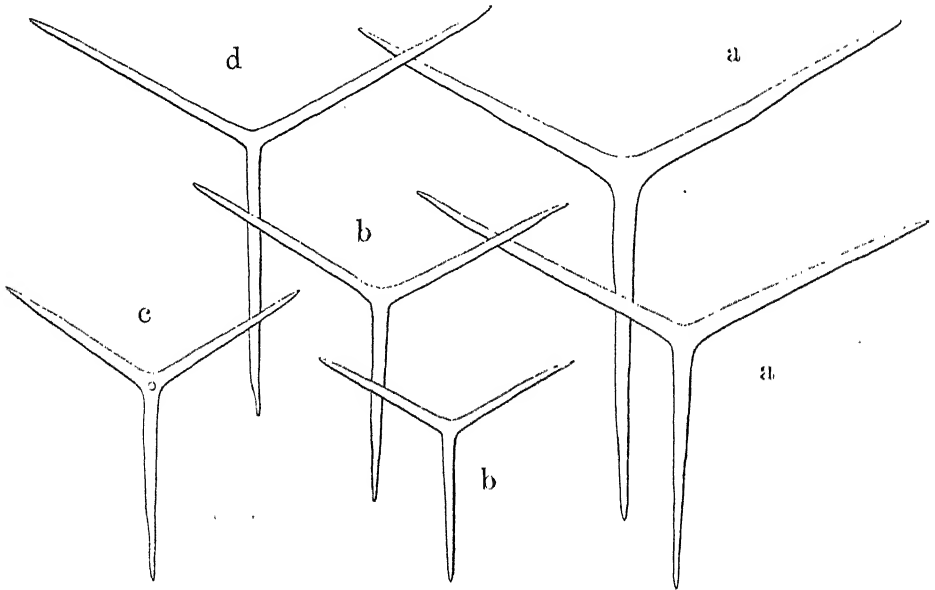
The Ascon-tubes are said to be arranged radially round the central pseudogaster though not in a regular manner and lie close to one another, but the anastomosis does not seem to occur. Proximally they communicate

<sup>1)</sup> DENDY, 1891, pp. 27, 30–32.

with the pseudogaster and terminate distally and blindly. Thus, they form the wall of the sponge and their blind ends are set in contact with one another, and are protected by special spicules, forming the outer surface of the sponge.

The skeleton of the pseudoderm consists of large triradiates, which are densely arranged in a few layers leaving spaces of angular outline for pseudopores. The walls of the Ascon-tubes are made up of an admixture of triradiates and quadriradiates. They are arranged in a thin confused layer, and the apical rays of the latter kind of spicule project into the gastral cavity. Triradiates are more numerous than quadriradiates. The lining membrane of the pseudogaster is composed mainly of triradiates disposed in a thin confused layer, and there may be added a small number of quadriradiates with their apical rays protruding into the pseudogaster.

Spicules (Text-fig. 1).—Triradiates of the pseudoderm (a) regular or subregular. All rays are generally straight, but are some-times more or less irregular in outline, being  $160\text{--}190\ \mu$  long and  $14\text{--}18\ \mu$  thick at the base.



Text-fig. 1. *Leucosolenia soyo*, n. sp.

a, Triradiates of the pseudoderm. b, Triradiates of Ascon-tubes. c, Quadriradiate of the same. d, Triradiate of the lining membrane of pseudogaster. (All  $\times 200$ ).

Triradiates of Ascon-tubes (b) regular or subregular with rays straight

and gradually sharply pointed,  $100-120\ \mu$  long and  $8-10\ \mu$  thick at the base.

Quadriradiates of Ascon-tubes (c) exactly the same as triradiates of the same save for the presence of an apical ray. The apical ray is slightly shorter and much thinner than the facial rays. It is almost straight, and stands vertically at the centre of the facial rays, being about  $80\ \mu$  long by  $4\ \mu$  thick.

Triradiates of the lining membrane of pseudogaster (d) regular. They are nearly similar to triradiates of the Ascon-tubes but on the whole larger, the rays reaching as much as  $160\ \mu$  in length.

Quadriradiates of the lining membrane of the pseudogaster are exactly similar to triradiates of the same membrane, except for the presence of a fine apical ray.

*Localities*.—Off Fudai, Rikuchiu (St. 61, Lat. N.  $40^{\circ} 3' 24''$ , long. E.  $142^{\circ} 11' 33''$ ); Near Sado Island (St. 600, Lat. N.  $38^{\circ} 33' 37''$ , long. E.  $138^{\circ} 21' 50''$ ).

*Remarks*.—In the external features and canal system, this species closely resembles *Leucosolenia amitsbo* HÔZAWA<sup>1)</sup> obtained from the Sagami Sea, Japan, but it may be easily distinguished from the latter by the character of the spicules. In the present species the triradiates forming the skeleton of the pseudoderm are much smaller than those of *L. amitsbo*, and are more thickly distributed. The meshes which are formed by these spicules for inhalant water are circular or oval with a smooth margin, while in *L. amitsbo* they are surrounded by an angular margin. The tri- and quadriradiates of the lining membrane of the pseudogaster have their rays distinctly shorter than those of the same membrane in *L. amitsbo*.

The difference in size between the pseudodermal triradiates and triradiates of Ascon-tubes is not so marked in the present species as in the case of *L. amitsbo*.

### Family Heteropiidae DENDY.

#### Genus GRANTESSA VON LENDENFELD.

#### 3. *Grantessa intusarticulata* (CARTER).

(Pl. I, fig. 3).

*Hypograntia intusarticulata*, CARTER 1886, p. 45.

*Hypograntia medioarticulata*, CARTER 1886, p. 46.

*Grantessa intusarticulata*, DENDY, 1892, p. 108; 1893, pp. 181, 201, Pl. XIII, fig. 18;

<sup>1)</sup> *Leucosolenia amitsbo*, HÔZAWA, 1929, pp. 283-285, Pl. XII, figs. 3, 4; Text-fig. 2.

DENDY and ROW, 1913, p. 753; HÔZAWA, 1916, p. 14, Pl. I, figs. 4, 5; Pl. II fig. 13, Text-fig. 3; 1929 p. 318; BRØNDSTED, 1926, p. 308; ROW and HÔZAWA, 1932, p. 775.

*Grantia intusarticulata*, BREITFUSS, 1897, p. 219.

This species is represented in the collection by a single specimen (Spec. No. P. 184; Pl. I, fig. 3) obtained from the Sagami Sea at Station 271 (Lat. N.  $34^{\circ} 58' 3''$ , long. E.  $139^{\circ} 35' 20''$ ; depth 82 m.).

It is a solitary tubular individual more or less irregular in contour, being broad near the attachment base and gradually narrowing towards the terminal osculum. It measures about 30 mm. in total length and 15 mm. in the greatest breadth. The thickness of the wall is about 1 mm. and the osculum measures about 5 mm. in maximum diameter.

With respect to the minor structure, i.e. canal system, skeleton and spiculation, it has been fully recorded by CARTER, DENDY, BRØNDSTED and myself, and thus it may be considered that no further details are needed here.

*Localities*.—Near Port Phillip Heads (CARTER, DENDY); Watson's Bay, Port Jackson (DENDY); Island Bay, Wellington, N. Z. (BRØNDSTED); Geraldton District, S. W. Australia (ROW and HÔZAWA); Misaki, Dôketsba (HÔZAWA); off Sunosaki, Sagami Sea.

*Remarks*.—This species was first described by CARTER (1886) as found in Australia, and, afterwards, it has been reported by DENDY (1892) and by ROW and HÔZAWA (1931) as found in the same locality and by BRØNDSTED as found in New Zealand (1926).

In 1916 I reported this species from Misaki and Dôketsba in the Sagami Sea, and this is the second case of the species being recorded as found in the sea of Japan. The specimen dealt with in the present paper was obtained off Sunosaki in the Sagami Sea.

Judging from its occurrence in Australia, New Zealand, and Japan, this species seems to be widely distributed.

#### Genus AMPHIUTE HANITSCH.

#### 4. *Amphiute ijimai* HÔZAWA.

(Pl. I, fig. 4)

*Amphiute ijimai*, HÔZAWA, 1916, pp. 33–38, Pl. I, fig. 9; Pl. II, fig. 17; Text-fig. 7; 1929, p. 313.

In the collection there is only a single specimen (Spec. No. P. 189, Pl. I, fig. 4) which was secured at Seno-umi in Suruga Bay (Station 278, Lat. N.  $32^{\circ} 42' 50''$ , long. E.  $138^{\circ} 30' 30''$ ; Depth 79 m.)

This single sponge is tubular, forcibly compressed laterally and with the basal parts torn off. The total length is about 55 mm. and the breadth near the lower end is about 27 mm. The breadth gradually diminishes towards the upper end, where an oval osculum of about 10 mm. maximum diameter opens. The wall is about 1 mm. thick in the lower parts but, becomes gradually thinner towards the osculum which is surrounded by a thin margin. The gastral cavity is very capacious, extending through the entire specimen.

The colour of the specimen under consideration is grey and looks very dirty owing to the fine sand attached to the sponge-surface.

In the other external features as well as in the minor structure of the interior the present specimen conforms very well to the type. Hence, it is considered that no further details need to be added here.

*Localities.*—Dôketsba, Sagami Sea (HÔZAWA); Seno-umi, Suruga Bay (St. 278. Lat. N.  $34^{\circ} 42' 50''$ ; long E.  $138^{\circ} 30' 30''$ ).

*Remarks.*—The genus *Amphiute* is characterized by the possession of colossal longitudinal oxea in both dermal and gastral cortices, in addition to the presence of subdermal pseudosagittal triradiates. At present this genus is represented by two species only i.e. *A. paulini* and *A. ijimai*. The first species which constitutes the type of the genus was described by HANITSCH having been obtained on the west coast of Portugal, while *A. ijimai* from Japan, was described and figured by myself the specimen having been secured at Dôketsba in the Sagami Sea.

The present record reports the occurrence of *A. ijimai* in Japan for the second time and on this occasion the specimen was obtained at Seno-umi in Suruga Bay, which lies west of Sagami Sea, being separated from the latter by the Izu Peninsula.

### Family Grantiidae DENDY.

#### Genus GRANTIA FLEMING.

#### 5. *Grantia glabra*, n. sp.

(Pl. I, fig. 5; text-fig. 2).

Three specimens of this species exist in the collection.

The first (Spec. No. P. 191; Pl. I, fig. 5), which is the largest, was obtained off Tsuchizaki, Ugo (St. 630. Lat. N.  $39^{\circ} 52' 45''$ , long. E.  $139^{\circ} 34'$ ) at a depth of 150 m.

The sponge represents a single type of a slightly laterally compressed tubular form, gradually narrowing towards the attachment base and showing



at the upper end an oval osculum, which is surrounded by a feebly developed collar. The dermal surface is smooth without any projecting oxea, while the gastral is distinctly echinated by the projecting apical ray of the gastral quadriradiates. It is 21 mm. long, and about 4 mm. broad near the distal end. The sponge wall is about 0.7 mm. thick. The osculum is about 1 mm. in maximum diameter.

The colour is greyish white, and the texture is fairly rigid.

The second and third specimens (Spec. Nos. 78 and 192) secured off Shioyazaki, Iwaki (St. 122. Lat. N.  $36^{\circ} 54' 30''$ , long. E.  $141^{\circ} 17'$ ; depth 161 m.) are much smaller than the first but as regards the microscopical structure they are absolutely identical.

As the subject of further description I select the first specimen.

Structure.—The canal system is of the syconoid type. The flagellate chambers are of an elongated sac-like shape, measuring about  $500\mu$  in length and about  $150\mu$  in diameter. They are usually simple without giving off any branches.

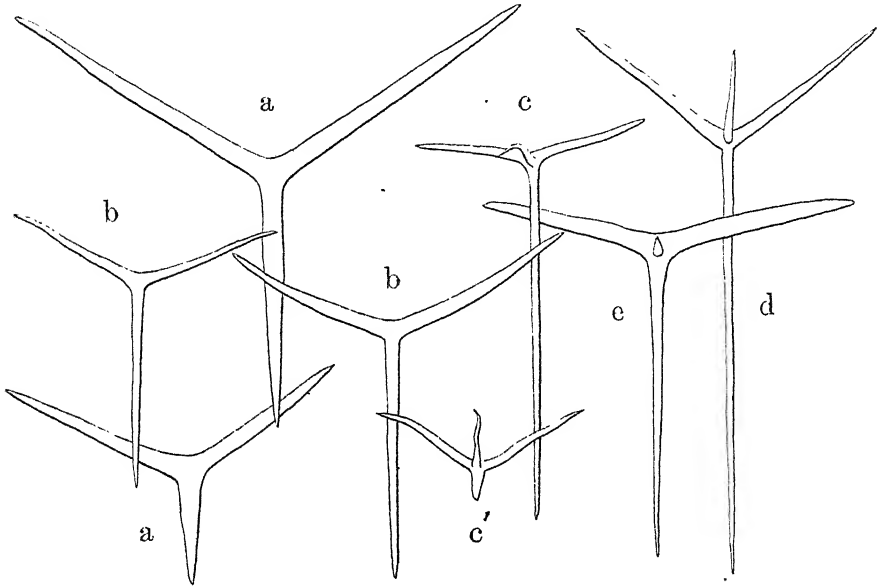
The dermal skeleton is composed of triradiates, which are tangentially but confusedly arranged in several layers. The tubar skeleton which is made up chiefly of triradiates is of the many-jointed articulate type. Here may be added the basal rays of the subgastral quadriradiates with apical rays projecting into the apopyle. The gastral skeleton forms a thin layer consisting mainly of gastral quadriradiates, of which the basal ray generally points towards the base, and the apical ray projects into the gastral cavity in oblique inclination towards the osculum. The oscular margin is composed chiefly of quadriradiates, with their basal ray running longitudinally and parallel with one another and the paired rays very strongly diverging. The apical ray is much shorter than that of the gastral quadriradiates.

Spicules (Text-fig. 2). Dermal triradiates (a) slightly sagittal with paired rays slightly longer than basal ray. All rays are of equal thickness and taper from base to sharp point. Basal ray straight, being  $70\text{--}150\mu$  long by  $14\text{--}20\mu$  thick. Paired rays slightly curved forwards, being  $110\text{--}170\mu$  long by  $14\text{--}20\mu$  thick.

Tubar triradiates (b) sagittal. Basal ray quite straight, longer and slightly thinner than paired rays, gradually tapering and sharply pointed, being about  $170\mu$  long by  $12\mu$  thick at the base. Paired rays widely divergent, and curved round the flagellate chamber, rather irregular in outline, and about  $120\mu$  long by  $12\mu$  thick.

Subgastral quadriradiates (c) strongly sagittal. Basal ray straight, standing vertically to the plain formed by the apical and paired rays, and

much longer than these rays, being about  $210\ \mu$  long and about  $10\ \mu$  thick at the base. Paired rays widely diverging and slightly curved, and about  $70\ \mu$  long by  $10\ \mu$  thick. Apical ray short slightly curved and, moreover, in most cases crooked, broad at the base, and very finely pointed at the end, being about  $30\ \mu$  long and about  $6\ \mu$  thick at the base.



Text-fig. 2. *Grantia glabra*, n. sp.

a, Dermal triradiates. b, Tubar triradiates. c, Subgastral quadriradiate. c', The same, facial view. d, Gastral quadriradiate. e, Quadriradiate of the oscular margin. (All  $\times 200$ ).

Gastral quadriradiates (d) sagittal. Basal ray much longer than paired rays, quite straight, tapering from base to the sharp point, being about  $250\ \mu$  long and about  $8\ \mu$  thick at the base. Paired rays almost straight, nearly equal or slightly differentiated in length, somewhat thicker than the basal ray, gradually tapering, sharply pointed, being about  $120\ \mu$  long and about  $10\ \mu$  thick at the base. Apical ray fairly well developed, longer than paired rays, slightly curved upwards and sharply pointed, measuring up to  $150\ \mu$  in length by about  $8\ \mu$  thick at the base.

Quadriradiates of oscular margin strongly sagittal. Basal ray straight, nearly uniformly thick for the greater part of its length, sharply pointed, being about  $200\ \mu$  long and  $10\ \mu$  thick at the base. Paired rays distinctly shorter and thicker than basal ray, slightly curved backwards, very strongly diverging, nearly uniformly thick for the greater part of their length, and

ending in an obtuse point. Apical ray much shorter than either basal or paired rays, being slightly curved and very sharply pointed and directed upwards.

*Localities*.—Off Tsuchizaki, Ugo (St. 630. Lat. N.  $39^{\circ} 52' 45''$ , long. E.  $139^{\circ} 34'$ ); off Shiroyazaki, Iwaki (St. 122. Lat. N.  $36^{\circ} 54' 30''$ , long. E.  $141^{\circ} 17'$ ).

*Remarks*.—This species may be easily distinguished from most members of the genus *Grantia* by the absence of oxeote spicules of any kind. It is closely related to *Grantia invenusuta* LAMBE<sup>1)</sup> from Davis Strait between Canada and Greenland. The present species corresponds with that species in external form and general anatomy, and in the absence of oxea of any kind, but it can be distinguished from the latter chiefly by the absence of gastral triradiates, by the presence of subgastral quadriradiates, by the character of the dermal triradiates, which have greater dimensions in comparison with the other spicules.

#### 6. *Grantia kujiensis*, n. sp.

(Pl. I, fig. 6; text-fig. 3)

Only a single specimen of this species is found in the collection (Spec. No. P. 52, Pl. I, fig. 6).

It was obtained at Station 64 (Lat. N.  $40^{\circ} 14' 50''$ , long. E.  $142^{\circ} 5' 10''$ ) at a depth of 150 m.

The sponge represents a solitary person of an oval form, narrowing towards the attachment base, and showing near the upper end an irregularly oval osculum about 2 mm. in diameter. The dermal surface is fairly hispid on account of the projecting oxea. The gastral surface looks nearly smooth to the naked eye, though it is punctated by minute exhalant apertures uniformly distributed. The gastral cavity is fairly large, but is traversed by the ingrowth of the gastral layer which forms an irregular meshwork.

The colour in alcohol is greyish white and the texture is fairly firm and elastic.

The specimen is about 12 mm. in length and 8 mm. broad in the broadest part. The sponge wall is about 1 mm. thick.

*Structure*.—The canal system is syconoid. The flagellated chambers are cylindrical, and usually not branched, measuring about  $500\ \mu$  long by  $150\ \mu$  thick. The dermal skeleton is weakly developed, and is scarcely distinguishable from the tubar skeleton. It is composed of triradiates

<sup>1)</sup> *Grantia invenusuta*, LAMBE, 1900, p. 32, Pl. VI, figs. 14, 14 a-f.

disposed irregularly in a small number of layers. The large oxea are placed at varying angles to the dermal surface, with their distal ends freely projecting on it, and with their proximal ends deeply intruding into the chamber layer. The tubar skeleton is of an articulate, though not very typical type, and is composed of triradiates in several rather confused layers. In the subgastral position there sometimes occur a small number of quadriradiates with their basal ray intruding into the chamber layer and with the apical ray projecting into the apophyle.

The gastral skeleton is made up of triradiates and quadriradiates, which are arranged tangentially in several but not numerous layers with the apical rays of the quadriradiates projecting into the gastral cavity. The skeleton of the meshwork, which is formed by the ingrowth of the gastral layer, and traverses the gastral cavity is composed of triradiates and quadriradiates exactly like those of the gastral skeleton. As regards the skeleton of the oscular margin, we have not noticed any peculiarities to be mentioned.

Spicules (Text-fig. 3).—Dermal triradiates (a) slightly sagittal, and the oral angle is slightly wider than the paired angles. Basal ray nearly straight, slightly longer than paired rays, being  $100\text{--}130\ \mu$  long and  $12\text{--}16\ \mu$  thick at the base. Paired rays subequal in length, generally straight with the exception on of a slight curvature near the base, gradually tapering and sharply pointed, being  $80\text{--}110\ \mu$  long and  $12\text{--}16\ \mu$  thick at the base.

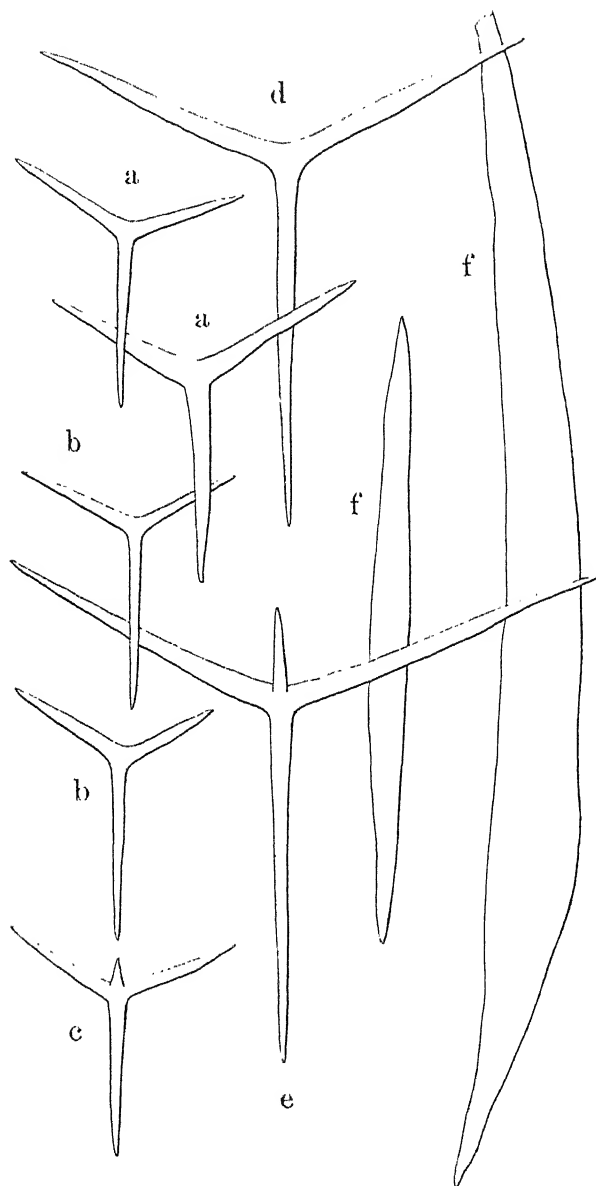
Tubar triradiates (b) almost like the dermal triradiates, but, on the whole, they have a wider oral angle and more slender rays than the latter. Basal ray is  $110\text{--}130\ \mu$  long by  $10\text{--}12\ \mu$  thick, and the paired rays are  $70\text{--}90\ \mu$  long and  $10\text{--}12\ \mu$  thick at the base.

Subgastral quadriradiates (c) nearly similar to the tubar triradiates with addition of an apical ray. This ray is much shorter than the facial rays, broad at the base but becoming thinner rather suddenly and ending in a very sharp point; it is about  $30\ \mu$  long and  $6\text{--}8\ \mu$  thick at the base.

Gastral triradiates (d) sagittal. Basal ray straight slightly longer than paired rays, gradually and sharply pointed, being about  $180\ \mu$  long and about  $16\ \mu$  thick at the base. Paired rays slightly curved at the base, and nearly straight in the remaining part, gradually and sharply pointed; is about  $150\ \mu$  long and about  $16\ \mu$  thick at the base.

Gastral quadriradiates (e) similar to gastral triradiates, differing only in the presence of apical ray. Apical ray distinctly shorter than facial rays, being slightly curved upwards, broad at base and narrowing distally

to terminate in a sharp point; is about  $70\ \mu$  long and about  $12\ \mu$  thick at the base.



Text-fig. 3. *Grantia kujiensis*, n. sp.

a, Dermal triradiates. b, Tubar triradiates. c, Subgastral quadriradiate. d, Gastral triradiate. e, Gastral quadriradiate. f, Large, oxea projecting from dermal surface. (All  $\times 200$ ).

Triradiates and quadriradiates which sustain the ingrowth from the gastral layer are almost the same as those forming the gastral skeleton.

Large oxea projecting from dermal surface (f) spindle-shaped, a little irregular in outline, generally broadest at a point nearer proximal than distal end, and tapering towards both pointed ends. They are more or less curved, and are of very variable length, 0.4–1.5 mm. long and 25–70  $\mu$  thick.

*Locality*.—Off Kuji, Rikuchiu (St. 64. Lat. N.  $40^{\circ} 14' 50''$ , long. E.  $142^{\circ} 5' 10''$ ).

*Remarks*.—I could not identify this form with any species already known, and, thus regarded, it represents a distinct species. Among the characteristic features above-mentioned the existence of the ingrowth from gastral layer into gastral cavity is most noticeable.

#### Genus LEUCANDRA HAECKEL.

##### 7. *Leucandra dura* HÔZAWA.

(Pl. I, fig. 7).

*Leucandra dura*, HÔZAWA, 1929, pp. 371–373, Pl. XXII, figs. 66–68, text-fig. 33.

Only a single specimen of this species exists in the collection (Spec. No. P. 190; Pl. I, fig. 7).

It was collected at Station 376 (Lat. N.  $34^{\circ} 33' 35''$ , long. E.  $138^{\circ} 7' 45''$ , depth 64 m.) which is situated near Ômae-zaki, Tôtômi. This sponge is in the shape of an irregularly rounded mass with a height of about 35 mm. and a maximum diameter of about 60 mm. It is broadly attached by the under surface, and its upper surface is strongly folded showing many lobose protuberances and furrows of various depths. There are four oscula, distributed on the upper surface. They are bare and vary in size, measuring 1–5 mm. in diameter.

The other characteristics in external features and in internal anatomy are exactly the same as those of the type specimen, and I have already given a detailed account on it in my paper mentioned above.

*Localities*.—Misaki (HÔZAWA); Off Ômae-zaki, Tôtômi (St. 376. Lat. N.  $34^{\circ} 33' 35''$ , long. E.  $138^{\circ} 7' 45''$ , depth 64 m.)

*Remarks*.—This species was first described by myself in 1929, the type specimen being obtained in shallow water in the neighbourhood of the Misaki Marine Biological Station. The present report notes the occurrence of this species in the sea of Japan for the second time. However, this time it was dredged at a depth of 64 m.

8. *Leucandra yuriagensis*, n. sp.

(Pl. I. fig. 8; text-fig. 4)

The single specimen (Spec. No. P. 107; Pl. I, fig. 8), on which this species is based, was dredged at a depth of 115 m. off Yuriage, Rikuzen (St. 102, Lat. N.  $35^{\circ} 10' 10''$ , long. E.  $140^{\circ} 36'$ ).

It is in the form of an irregular cylindrical tube. The broader base is provided with a number of globular processes and depressions, while the upper parts become gradually narrower towards the distal end, where an osculum opens. The total length is about 60 mm. and the breadth is 45 mm. in the broadest part. The wall is thickest in the basal part, measuring about 3 mm., and becomes gradually thinner towards the osculum, which is surrounded by a very thin margin. The osculum is irregularly elliptical with the greater diameter of about 10 mm. The dermal surface looks nearly smooth being without any projecting spicules, while the gastral surface is slightly hispid, and is perforated by apertures of exhalant canals of variable sizes, measuring from 0.3 to 0.8 mm. in diameter.

The gastral cavity is very spacious, its form corresponding to that of the specimen.

The colour in alcohol is white. The texture is hard and fairly elastic.

Structure.—The canal system is of the leuconoid type. The flagellate chambers are rather thinly distributed in the chamber layer, and are irregularly scattered. They are generally of an oval shape measuring up to  $70\ \mu$  in the longer diameter. Underneath the dermal cortex there are subdermal cavities in fairly uniform distribution, and the inhalant canals start from these cavities. The chamber layer is also traversed by exhalant canals varying in thickness.

The wall of the sponge is supported by three distinct skeletal layers, namely, a dermal skeleton, a tubar skeleton, and a gastral skeleton. The dermal skeleton is rather thin, and is composed of large and small triradiates placed tangentially in a small number of confused layers. The tubar skeleton, e.i. the skeleton of the chamber layer consists of triradiates, which are thickly and irregularly set together. The walls of the larger exhalant canals are lined with quadriradiates with the basal ray usually pointing away from the gastral surface, and with the apical ray projecting into the canal. The gastral skeleton is nearly equal to the dermal in thickness, being fairly well distinguished from that of the chamber layer. It is composed of a dense reticulation of tangential tri- and quadriradiates. There may be in addition large tangential triradiates thinly distributed

and microxea, which are fairly thickly distributed, and which are placed tangentially but without any definite orientation. The thin oscular margin contains tri- and quadriradiates, both of which are very closely and regularly set together with the basal ray pointing towards the sponge base, and with strongly divergent paired rays. There also occur sometimes a small number of large triradiates and microxea.

Spicules (Text-fig. 4).—Larger dermal triradiates (a) equiradiate and equiangular. All rays straight, gradually and sharply pointed being  $410\ \mu$ – $1.25\text{ mm.}$  long and  $40$ – $130\ \mu$  thick at the base.

Smaller dermal triradiates (b) slightly sagittal with rays of equal thickness and disposed slightly convexly towards the outer side. Basal ray straight, not strongly differentiated in length from the paired rays; being  $140$ – $400\ \mu$  long and  $14$ – $30\ \mu$  thick at the base; paired rays gently curved forwards in greater part of the basal and nearly straight in the distal part; being  $100$ – $270\ \mu$  long and  $14$ – $30\ \mu$  thick at the base.

Triradiates of the chamber layer regular or subregular, very large but variable in size. They are exactly similar to the larger triradiates found in the dermal cortex.

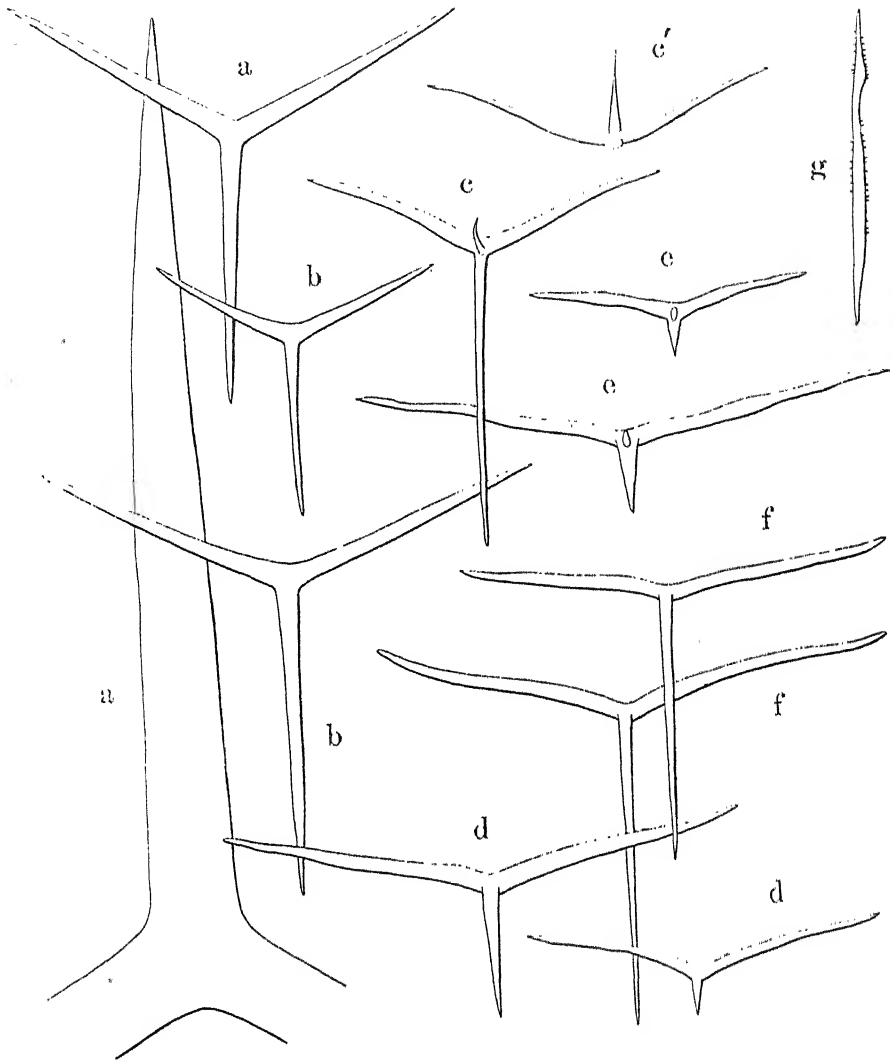
Quadriradiates of the larger exhalant canals (c, c') sagittal. Basal ray straight, sharply pointed, slightly longer and thinner than paired rays; being about  $420\ \mu$  long and  $20\ \mu$  thick at the base. Paired rays more or less curved round the exhalant canals, and in facial view, they show a double curvature, forwards in the greater part of the basal and slightly backwards in remaining parts, it is about  $240\ \mu$  long and  $28\ \mu$  thick at the base. Apical ray shorter and thinner than facial rays, slightly curved and very finely pointed; it is  $50$ – $200\ \mu$  long and about  $14\ \mu$  thick at the base.

Larger gastral triradiates regular or sub-regular, exactly similar to those of the dermal cortex and of the chamber layer.

Smaller gastral triradiates (d) strongly sagittal. Rays lying nearly in one plane. Basal ray much shorter and slightly thinner than paired rays, almost straight and sharply pointed, being  $60$ – $180\ \mu$  long and  $18$ – $20\ \mu$  thick at the base. Paired rays very widely divergent, slightly curved backwards in the greater part of the basal, and nearly straight in remaining parts, generally more or less irregular in outline, being  $230$ – $370\ \mu$  long and  $26$ – $30\ \mu$  thick at the base.

Gastral quadriradiates (e) exactly similar to the smaller gastral triradiates except in the presence of an apical ray. This is sharply pointed and is slightly curved; it is  $50$ – $110\ \mu$  long and about  $14\ \mu$  thick at the base.



Text-fig. 4. *Leucandra yuriagensis*, n. sp.

a, Larger dermal triradiates. b, Smaller dermal triradiates. c, Quadriradiate of the larger exhalant canal. c', The same, facial view. d, Smaller gastral triradiates. e, Gastral quadriradiates. f, Triradiates of the oscular margin. g, Gastral microxea. (a-f  $\times 100$ ; g  $\times 500$ ).

Triradiates of the oscular margin (f) strongly sagittal. Basal ray straight, finely pointed, generally longer and sometimes shorter than paired rays. Paired rays widely diverging, mostly doubly curved, first backwards then forwards ending in a rather bluntly pointed end. They are nearly

uniformly thick in their greater length. In an example of the spicule the basal ray and paired rays measured respectively  $270\ \mu$  and  $260\ \mu$  in length by  $14\ \mu$  and  $20\ \mu$  thick at the base.

Quadriradiates of the oscular margin exactly the same as the triradiates of it but with a short apical ray.

Gastral microxea (g) usually slightly curved, broadest in the middle parts, and tapering towards both ends, of which one is sharply pointed and the other forms a hastate point. The sides of the spicule are provided with a number of very fine spines. An example of the spicule measured  $90\ \mu$  long and  $4\ \mu$  thick in the broadest parts.

*Locality*.—Off Yuriage, Rikuzen (St. 102, Lat. N.  $35^{\circ} 10' 10''$ , long. E.  $140^{\circ} 36'$ ).

*Remarks*.—In external form as well as in internal features, this new species closely resembles *Leucandra pacifica* HÔZAWA<sup>1)</sup> from Dôketsba in Sagami Sea. But there are some peculiarities in spiculation, by which the present species may be easily distinguished from that species. The peculiarities are as follows :

- 1) The presence in the present species of gastral sagittal triradiates which seem to be entirely wanting in *L. pacifica*.
- 2) In *L. pacifica* there are slender hair-like oxea projecting from the dermal surface. But they are entirely wanting in the present species.
- 3) The apical ray of the gastral quadriradiate spicule is more strongly developed in *L. pacifica* than in the present species.

#### LIST OF LITERATURE REFERRED TO.

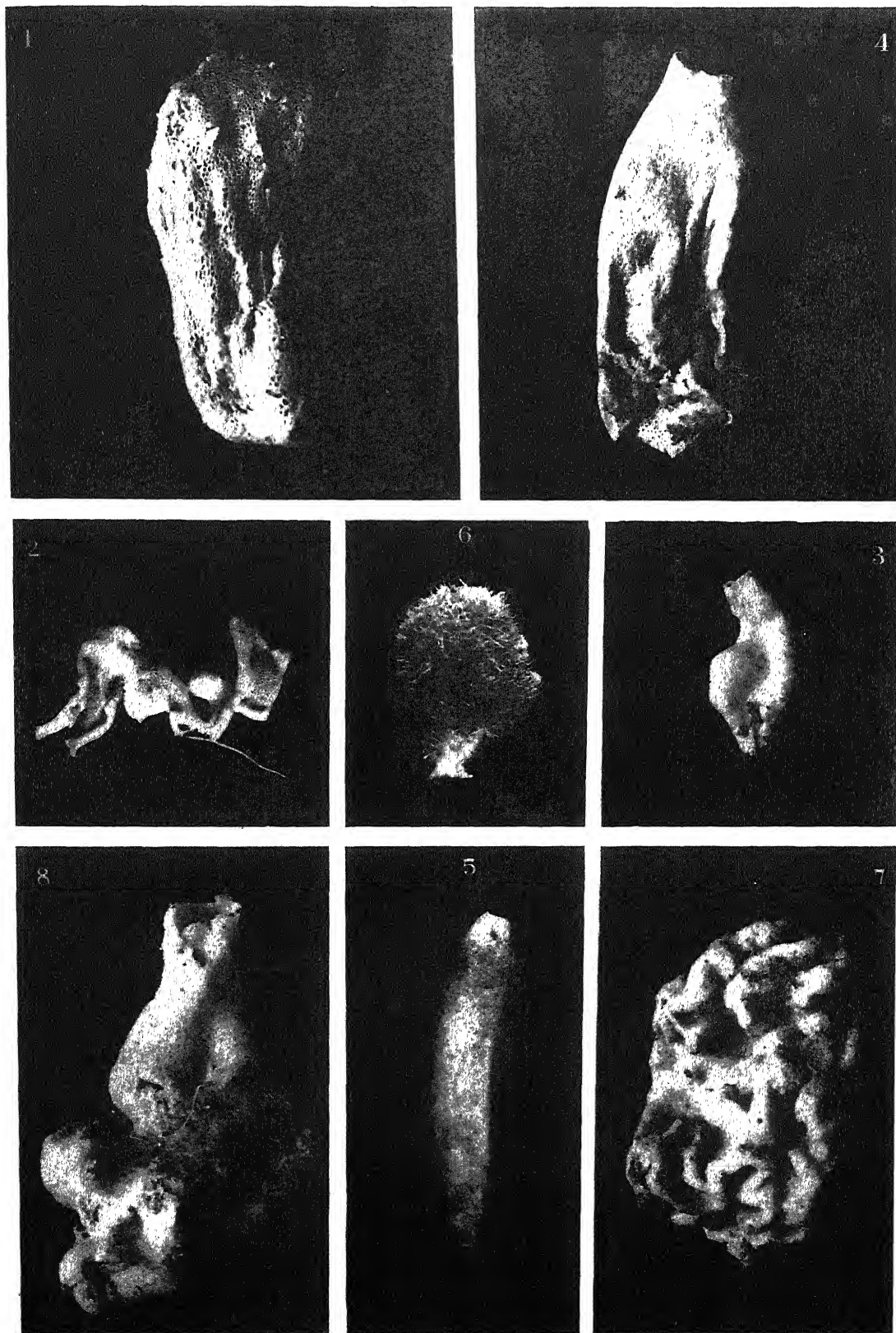
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<sup>1)</sup> *Leucandra pacifica*, HÔZAWA, 1929, pp. 368-370 Pl. XXI, figs. 63, 64; text-fig. 32.

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#### EXPLANATION OF PLATE I.

- Fig. 1. *Leucosolenia canariensis* MICHLUCHO-MACLAY.  $\times 1$ .
- Fig. 2. *Leucosolenia soyo*, n. sp.  $\times 1$ .
- Fig. 3. *Grantessa intusarticulata* CARTER.  $\times 1$ .
- Fig. 4. *Amphiute ijimai* HÔZAWA.  $\times 1$ .
- Fig. 5. *Grantia glabra*, n. sp.  $\times 3$ .
- Fig. 6. *Grantia kujiensis*, n. sp.  $\times 3$ .
- Fig. 7. *Leucandra dura* HÔZAWA.  $\times 1$ .
- Fig. 8. *Leucandra yuriagensis*, n. sp.  $\times 1$ .



S. HÔZAWA: Calcareous Sponges of Japan.



CONTRIBUTIONS TO THE PHYSIOLOGY OF THE  
HEART OF OYSTER.  
IV. THE ACTION OF ADRENALINE ON THE ISOLATED  
HEART OF OYSTER.\*

By

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(With 6 text-figures)

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I. INTRODUCTION.

The physiological actions of adrenaline have been studied by numerous investigators since the classical experiments of OLIVER and SCHAEFER (1895). It is generally known that, in mammals, birds, reptils and amphibia that adrenaline seems to stimulate the sympathetic nerve-endings. These effects ELLIOT (1905) named the 'sympathomimetic theory'. But this theory could not always be applied to the lower vertebrates such as fishes, not to invertebrates such as crustacea and mollusks; for instance, ELLIOT (1912) was unable to obtain any response in the heart of the crayfish, and also HOGBEN (1927) described the same result in the heart of the snail. CARLSON (1905), on the other hand, stated that adrenaline has an excitatory action both on the cardiac ganglion and the myocardium of the neurogenic heart of *Limulus*, and he stated that the action of adrenaline on the *Limulus* heart is identical with that on the mammalian heart, and the results on *Limulus* furnish a clue to the interpretation of the point of action of the drug in the vertebrate heart, since the ganglion and ganglion-free part of the heart react to adrenaline in the same manner. MACDONALD (1925) has studied the action of adrenaline on the perfused heart of the dog-fish, the phenomena described by him are puzzling; a characteristic preliminary inhibition in the normal rate was followed by an increase in the amplitude which was sometimes associated with acceleration. HOGBEN and HOBSON (1924) described a pronounced increase in the tone of the muscle and acceleration of the cardiac rhythm in the crab, *Maia squinado*, tonic contraction of the heart muscle of the Lamellibranch,

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\* Contributions from the Marine Biological Station, Asamushi, Aomori-ken. No. 97.

ecten, and the crop musculature of the Gastropoda, *Aplysia* and Polychaeta, *Lophrodite*, with dilution of 1:50,000 or even smaller quantities. TSUNODA (1923) stated that the action of adrenaline on the heart of the oyster always accelerates the auricle; on the other hand, the effect on the ventricle is inhibitory. Sometimes it is affected acceleratively and sometimes it firstly inhibits and then reacts acceleratively. He continued his statement that the effects of adrenaline vary in different individuals, but the concentration of adrenaline over 1:10,000 always reacts on the heart of the oyster. Recently BAIN (1929) has reported the following results of the action of adrenaline on the isolated crustacean heart; adrenaline produces a marked acceleration of the rhythm and an increase in the tone of the heart-muscle in *Maia*, *Cancer* and *Carcinus*; and, in *Cancer*, in addition to the above effects, there is a pronounced increase in the amplitude of beats. He continued his experiments and shows that the action of adrenaline is not reversible. In the lowest dilution in which it gives any effect, that effect is excitatory.

The present investigation attempts to examine the phenomena of the isolated heart of the oyster by adrenaline chloride quantitatively.

## II. MATERIAL AND METHOD.

The material employed in these experiments was *Ostrea circumpicta* PILSBRY. The heart is completely isolated from the body; the method of isolation of the heart from the body has been already described and therefore, the reader is referred to my former report regarding this subject (1927).

The isolated heart of the oyster which normally pulsates in sea water is transported to various concentrations of adrenaline chloride solution, diluted with sea water and the effects are observed by the kymographic method. The standard adrenaline chloride used in these experiments is diluted to 1:1,000 solution.

## III. EXPERIMENTAL RESULTS.

The heart of the oyster which normally pulsates in sea water is transported to the various concentrations of adrenaline chloride, certain effects show in certain concentrations. The summarized results are as follows.

I. To 300 c.c. of sea water are added 0.1 c.c., 0.2 c.c. and 0.3 c.c. of adrenaline chloride (1:1,000) and the effects are observed. In such concentrations, the heart shows no response at all, and pulsates normally.

More adrenaline (0.5 c.c.-1.0 c.c.) is added by aid of a pipette. In this solution, some individuals respond to it and show an increase in tone of muscle and acceleration of the cardiac rhythm, but these phenomena are invariable in each individual. Some of them do not respond and pulsate normally and are almost indifferent to the drug.

II. The quantity of adrenaline is increased to 2 c.c.-5 c.c. in 300 c.c. sea water. The results are similar to the former one; some of them respond to it and show the increase in tone of muscle and acceleration of pulsation, and, on the other hand, some individuals do not respond.

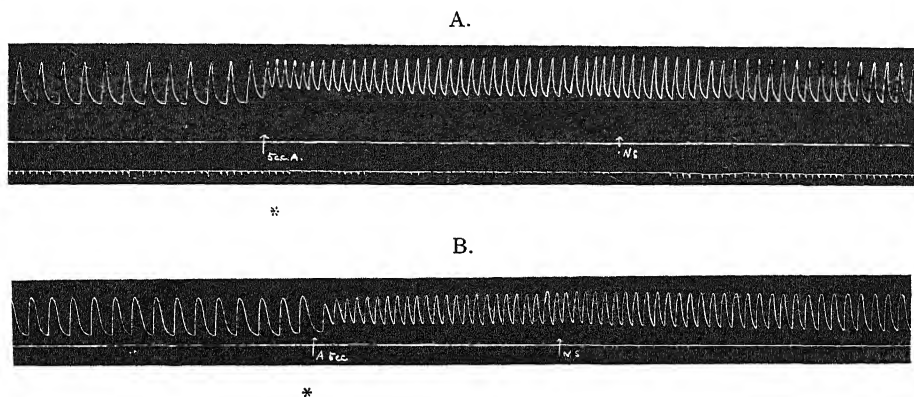


Fig. 1. A. The action of adrenaline on the heart. Temperature 22°C.

B. The action of adrenaline. Temperature 22°C.

\* 300 c.c. sea water +  $\frac{1}{1000}$  adrenaline chloride 5 c.c. N. S., Normal sea water.

III. By increasing the quantity of the drug to 10 c.c. in 300 c.c. or 250 c.c. of sea water, the results are characteristic as stated in regard to the heart of *Pecten* by HOGBEN and HOBSON; the heart suddenly increases in tonic contraction, and pulsation becomes very weak and appears to stop as in the following figures.

The phenomenon gradually disappeared and then the tone decreased gradually and the pulsation contraction became more vigorous and the number of pulsations increased, about five minutes after, the tone and the pulsation number became normal. The reaction of the drug to the heart of the oyster is reversible. (But the time of recovering varies in different individuals).

IV. The strip of the ventricle also shows a similar response and the concentration of the drug to the strip seems lower than for the isolated whole heart. The following figure shows the effect on the strip of the



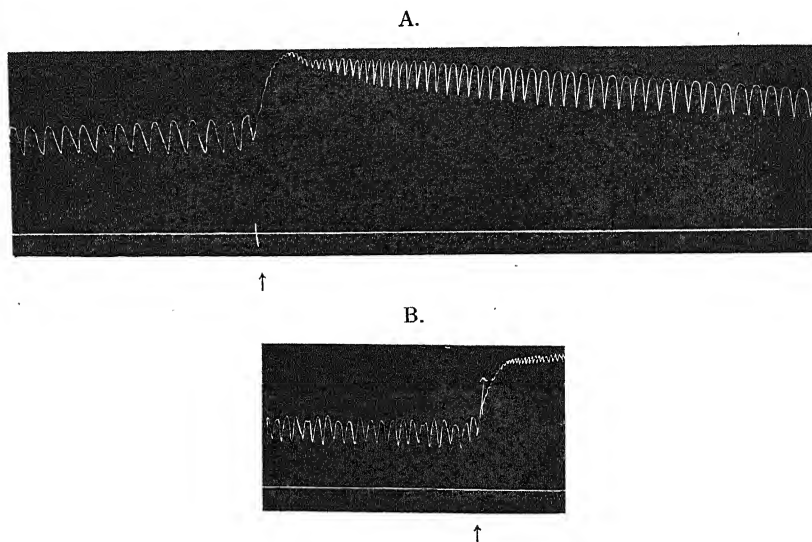


Fig. 2. The action of adrenaline chloride on the heart,

A. 250 c.c. sea water +  $\frac{1}{1000}$  adrenaline chloride 10 c.c., showing the heart suddenly increases in tonic contraction.

ventricle; the solution being 300 c.c. sea water +  $\frac{1}{1000}$  adrenaline chloride 1 c.c. But this effective concentration varies in different individuals.

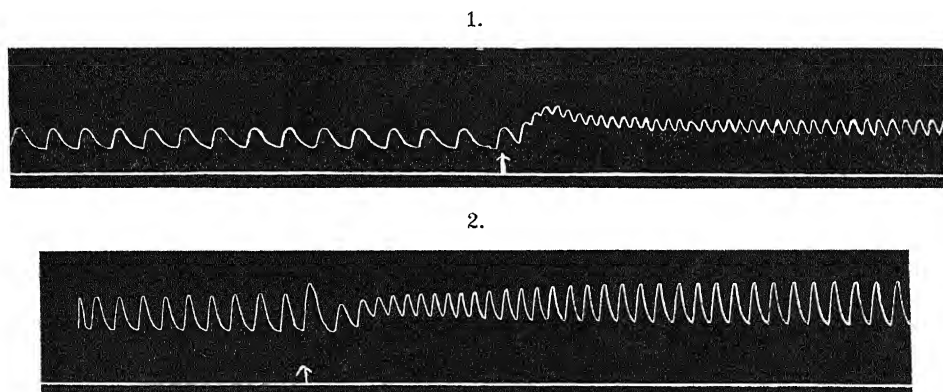


Fig. 3. The action of adrenaline on the strips of ventricle,

300 c.c. sea water +  $\frac{1}{1000}$  adrenaline chloride 1 c.c..

V. The isolated ventricle and auricle pulsate well in normal sea water as stated in my former report. These effects of the drug seem to be almost the same as in the other experiments mentioned. There is scarcely

any difference between the effects of the drug on the ventricle and auricle. Merely the effect of the concentration varies individually, though it appears that the auricle is more sensible than the ventricle from the standpoint of statistical results.

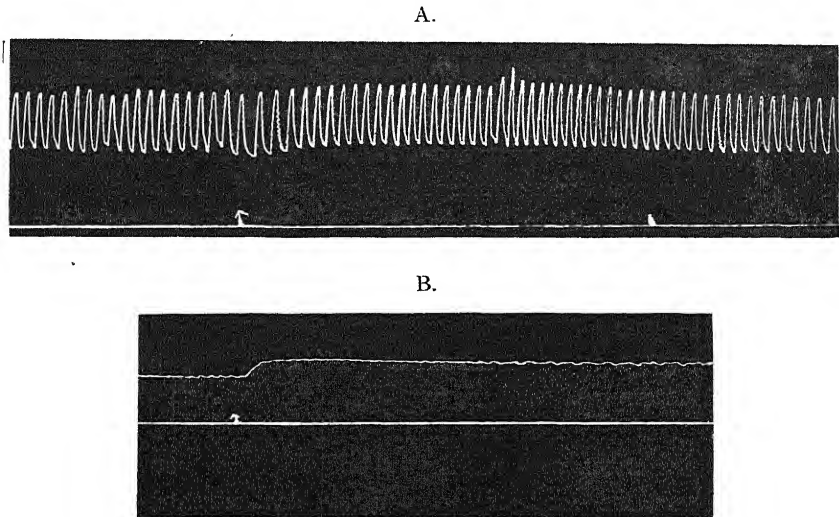


Fig. 4. A. The action of adrenaline chloride on the ventricle alone.

B. The action of adrenaline chloride on the auricle alone.

VI. To examine the automaticity of the heart after being treated with adrenaline and to ascertain whether or not it is affected by temperature. This experiment is only a preliminary one. The heart after being treated with the following drug solution, 150 c.c., sea water +  $\frac{1}{1000}$  adrenalin chloride 5 c.c., and returned to normal sea water and heated gradually with the alcohol lamp and then observed, the pulsation stops and initial heat rigor occurs. The results are as in the figure, showing scarcely any effects.

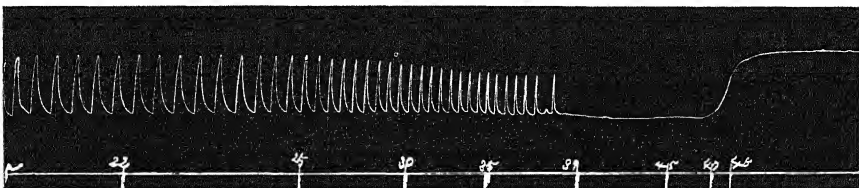


Fig. 5. Effect of temperature after treated with adrenaline and gradually heated with alcohol lamp.

## IV. GENERAL CONSIDERATION.

These experiments on the effect of adrenaline on the heart of the oyster show similar results as these on the heart in other Lamellibranch and in crabs and on some musculatures in Polychaeta.

According to BAIN (1929) the effects of adrenaline on the heart of the crabs (*Maia*, *Cancer*, *Carcinus*) occurred about four or five seconds after the admission of adrenaline (typical experiment;  $\frac{1}{40000}$  adrenaline solution) and there is a marked acceleration of the heart rate, the frequency of the rhythm increasing from 17 to 74 per minute, this being accompanied by a progressive increase in the tone of the muscle. In the oyster, the heart responds to the drug after a few seconds and does not show such an increase of pulsation; but as it will be seen in the figures, the frequency increases to much more than normal and the musculature also increases in tone.

The minimum effective concentration of adrenaline on the crab's heart was not determined in detail by BAIN, but he stated that in a dilution of 1 in 100 millions the excitation was just perceptible, while with 1 in 10 millions the effect was sufficiently obvious. As mentioned in the above experiments, the effect of the minimum concentration on the heart of the oyster varies in different individuals. The following is apparently about the minimum effective concentration; 300 c.c. sea water and  $\frac{1}{1000}$  adrenaline chloride 0.5 c.c.

HOGBEN and HOBSON who describe a pronounced increase in tone and acceleration of the cardiac rhythm of the crab (*Maia*, *Pecten*, and the crop musculature of *Aplysia*, and also *Aphrodite*, with dilution of 1:50,000 on even smaller quantities. According to the results of WYMAN and LUTZ (1931), "Adrenaline chloride solution added to the immersion fluid to make a concentration of 1 in 100,000 in the case of *Cucumaria*, usually caused a progressive decrease in tone, amplitude, and rate of the cloacal strip, with a final cessation of beat. In a few case a concentration of 1 in 200,000 produced a typical effect, but usually this dose affected only one or two of the three factors. In the case of *Sticopus* 1 in 50,000 was the effective dose, the type of response being the same as in *Cucumaria*".

In these experiments, I have not employed the perfusion method and therefore, it seems that the effective concentration on the oyster heart is different from the results stated by the above authors; consequently the action of adrenaline on the heart is due to the penetration quantities of the drug into the heart from the surrounding solution.

It is worth noting that sometimes the continuous increase of the drug produces no effect whatever, and in the case of the heart of the oyster

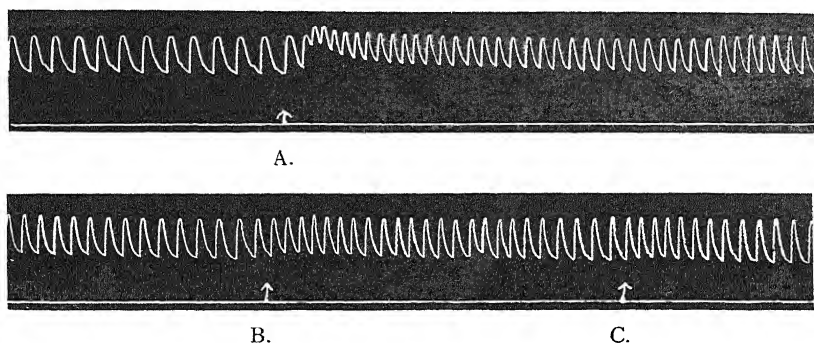


Fig. 6. The action of adrenaline chloride. Showing the continuous increase of the drug: A. 300 c.c. sea water +  $\frac{1}{1000}$  adrenaline 5 c.c.

B. " + " " 10 c.c.

C. " + " " 20 c.c.

it was not found possible to obtain an irreversible type of response, such as can be obtained from the crab's heart as stated by BAIN.

It is general, in *mammals* both auricles and ventricles are affected by adrenaline. But in the bird (pigeon) and reptile (tortoise) according to ELLIOT (1905), only the auricles are excited. PATON (1912) conforms ELLIOT's findings that adrenaline does not excite the ventricle of the bird and he stated that there is no augmentor nervous mechanism in connection with the avian ventricle. On the other hand, several investigators have described the augmentation and acceleration of the isolated heart of the frog with adrenaline.

TSUNODA reported that the action of adrenaline on the heart of oyster always affected the auricle acceleratively and on the ventricle is inhibitory. Sometimes it is affected on the ventricle acceleratively and sometimes it firstly inhibits and then reacts acceleratively.

The results of my experiment fail to show a difference in the heart of the oyster. The ventricle and the auricle are both invariably accelerated. Whether the action of adrenaline on the heart firstly affects the nervous system and then the cardiac muscle, or whether it affects the cardiac muscle only is a most important question. CARLSON (1905) stated that adrenaline has an excitatory action both on the cardiac ganglion and the myocardium of *Limulus*. Recently OKA (1932) determined the innervation of the heart of the oyster physiologically; the heart is provided with

accelerator and inhibitor nerves. But the histology of the nerves has not been clearly understood. Therefore, whether the effect of adrenaline affects firstly the ending of this accelerator nerve or directly affected the muscle itself is important.

Though HOGBEN (1926) stated in his book that "One may question whether much importance should be attached to the action of adrenaline as an indication of the existence of sympathetic innervation, an argument which has been widely used in physiological literature", it is of considerable value in speculation concerning the organisation of the neuro-muscular mechanism in the oyster heart. No special attention of the hydrogen concentration has been taken throughout the experiment, but determinations of the pH of the immersion fluid were made each time by the colorimetric method. The pH of the sea water at the beginning of an experiment was about 8.2. Addition of the usual dose of adrenaline chloride solution lowered it by 0.2 to 0.5 (Because sea water is a powerful buffer solution). I think that changes of pH of these magnitudes have not much effect on the preparation. (The effect of pH on the pulsation of the oyster heart is of interest and importance. My experiments are not yet completed them.)

Since adrenaline chloride solution contains some slight amount of chloretone, it is necessary to control tests for this substance, but owing to lack of time, I have been unable to test this substance on the heart. (According to WYMAN and LUTZ, concentrations of chloretone up to eight times the amount present in the largest dose of adrenaline chloride were found to be ineffective.)

I prefer to present these results merely as examples of the action of certain chemical substances on an autonomous and presumably neuro-muscular organ in the heart of the oyster; and to regard such actions as dependent on the nature of the chemical and physical constitution of the materials participating in the excitatory processes.

I am waiting for a more thorough understanding of the histological and morphological features of the heart of oyster especially with reference to the innervation and distribution of the nerve cells.

## V. CONCLUSION.

The heart of the oyster when affected by adrenaline always accelerates its pulsation and increases the tone of muscle. The minimum effective concentration of the drug varies in different individuals, but it is apparently

as follows ; 300 c.c. sea water +  $\frac{1}{1000}$  adrenaline chloride 0.5 c.c. There is no great difference between the effects of adrenaline on the ventricle and the auricle. The effect of adrenaline is a reversible reaction in the heart of the oyster and has no effect on the initial heat rigor of the cardiac muscle. It is highly important to study in the future the distribution of the nerve cells in order to scrutinize the possible relationship of the nervous system to the effects of adrenaline on the heart of oyster.

I wish to express my sincere thanks to Prof. S. HATAI for his valuable suggestions and criticisms during the course of this work.

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# ON THE SURFACE pH OF THE NORTH PACIFIC OCEAN.

By

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[With 1 text-figure.]

[Received November 10, 1932.]

Although the pH of the Pacific Ocean has been studied by MAYER<sup>1,2)</sup> and Iro<sup>3)</sup> it was thought worth while to make some additional observations. In order to rule out diurnal variations due to photosynthesis all observations were made at noon each day on water dipped from the surface by means of a bucket through a porthole of the Asama Maru, N. Y. K., between San Francisco and Yokohama. On March 29 no sample was taken since we were in Honolulu Harbor, and on April 2 the date line was passed before noon.

The pH determinations were made by means of test tubes 25 mm. diameter filled with buffer solutions and colored with thymol blue according to the method of McCLENDON, GAULT and MULHOLLAND<sup>4)</sup> and checked by means of the BAUSCH and LOMB, micro colorimeter and several indicators<sup>5)</sup>.

These indicators were checked by means of the hydrogen electrode of Dr. S. NOMURA in Sendai and of Mr. E. SAWANO in Asamushi.

The chlorine titrations were made by means of silver nitrate, using dichloro-fluorescein as indicator. The silver nitrate was standardized by means of standard sea water from the Oceanographic Institute at Kobe.

The results are given in the accompanying map and the following table.

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<sup>1)</sup>Proc. Natnl. Acad. Sci., 3:548 (1917).

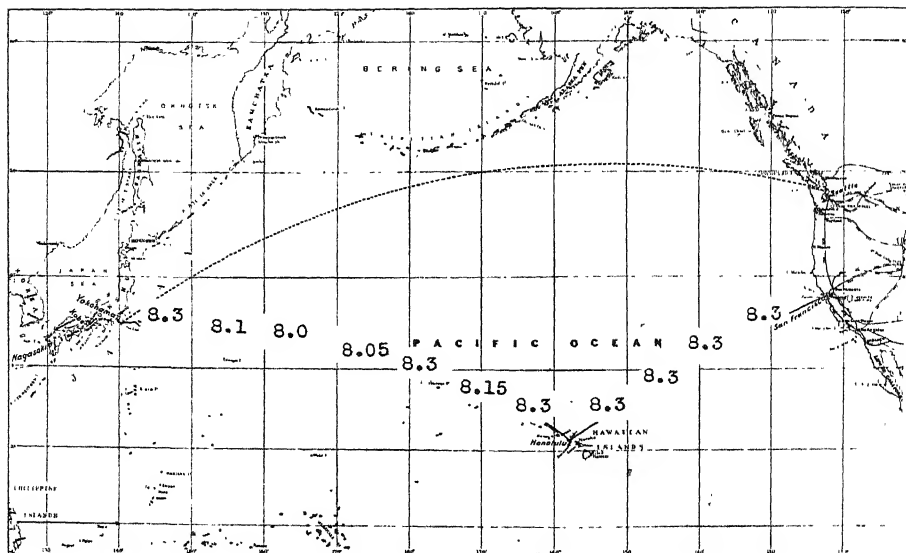
<sup>2)</sup>Proc. Amer. Phil. Soc., 58:150 (1915).

<sup>3)</sup>Records of Oceanographic Work in Japan, 1:90 (1928-9).

<sup>4)</sup>Papers of Dept. Mar. Biol. Carnegie Inst., 11:21 (1917).

<sup>5)</sup>J. F. McCLENDON: Series of Practical Chemistry. I. E. (實驗化學講座別刷)





Mar.		Lat. N.	Long. W.	Temp.	Cl	Salinity	pH
	Noon.						
25	„	35° 13'	130° 24'	15	18.1	33.5	8.3
26	„	32° 2'	138° 23'	17.5	18.7	34.5	8.3
27	„	28° 25'	145° 51'	20.5	18.8	35.2	8.2
28	„	24° 20'	152° 49'	23	19.0	35.3	8.3
30	„	24° 4'	162° 15'	22	19.3	35.4	8.3
31	„	27° 17'	170° 43'	20	19.2	35.5	8.15
Apr. 1	„	30° 3'	178° 56'	18	19.0	35.4	8.3
	date line		Long. E.				
3	„	32° 13'	172° 30'	17	18.9	35	8.05
4	„	33° 44'	163° 44'	17	18.9	34.6	8.00
5	„	34° 45'	154° 52'	17	18.9	34.6	8.10
7	„	35° 1'	145° 29'	17	--	34.8	8.30

# CHROMOSOME MORPHOLOGY IN *SMILACINA JAPONICA*.

By

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(With 8 text-figures)

(Received November 16, 1932)

The material for this study was obtained in the grounds of the Asamushi Marine Biological Station of our University at Sendai. Asamushi being situated near the northern limits of Honshu, many plants which, near Sendai are found only in mountain regions, grow there abundantly along the seashore. One example is *Smilacina japonica*, the plant which is to be dealt with in the present paper.

The fixing fluids, NAWASCHIN's and BOUIN-DUBOSCQ's were equally successful in the case of this plant. For the fixation of the pollen mother cells, however, the material was first dipped for about half a minute in CARNOY's fluid; this seems to be a most effective method for the fixation of this part of the plant.

Staining was carried out entirely according to NEWTON's gentiana violet-iodine method, which was decidedly better than HEIDENHAIN's iron alum haematoxylin. The materials were sectioned in paraffin as usual the smear method was not used.

Several species of *Smilacina* have already been investigated cytologically by McALLISTER (1909, 1913), LAWSON (1911, 1912), and WOOLERY (1915).

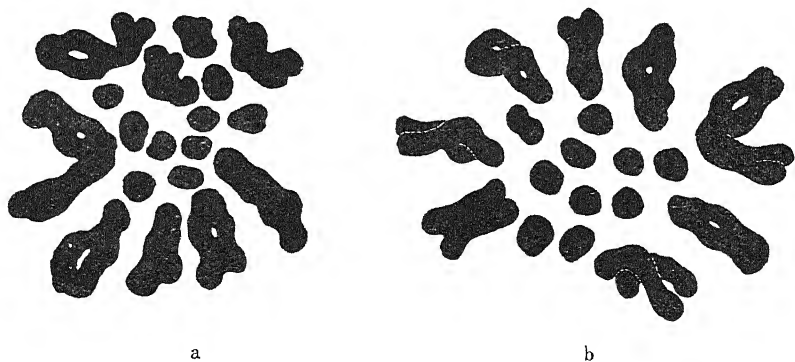


Fig. 1, a, b.—Polar views of 1st division in pollen mother cells.  $\times 1760$ .

But, hitherto, we have no exact knowledge concerning the chromosome morphology of this genus.

The chromosomes of *Smilacina japonica* are relatively large, and among them there are distinct differences in size and shape. Text-fig. 1, a and b are the polar views of the heterotypic mitosis in the pollen mother cells of this plant. The total number of chromosomes in this mitosis is eighteen, of which ten are much smaller than the remaining eight. The latter are generally situated in the periphery of the nuclear plate. Although at this stage it is rather difficult to distinguish the special characteristics of each of the larger chromosomes, one of these is remarkably large and is easily detected in each nuclear plate. This chromosome will be designated as A.

Text-fig. 2, a and b, show the side views of the anaphase of the same mitosis. Here only the eight larger chromosomes are separately depicted,

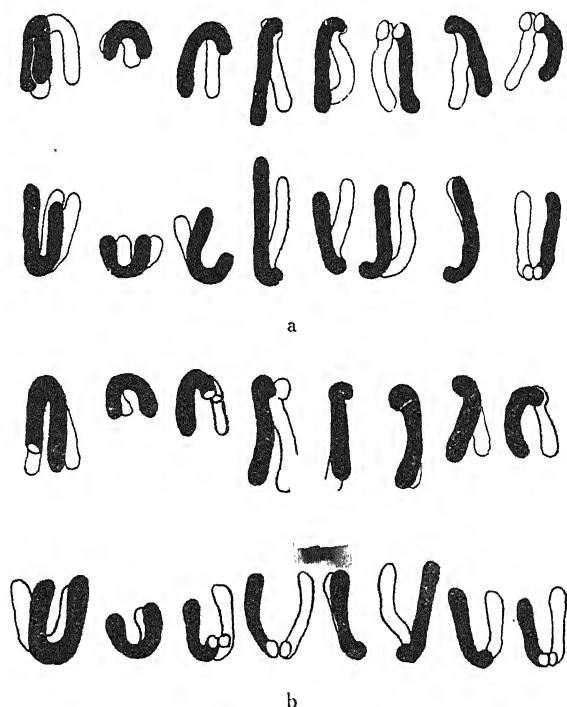


Fig. 2, a, b.—Large chromosomes in anaphase of 1st division of pollen mother cells.  $\times 1760$ .

to illustrate the size- and shape-differences clearly. The longitudinal splitting of each chromosome is already completed, and its two halves are seen widely separated. The chromosome A, which was already distinguishable

in the former stage is equally remarkable in this stage. As the figure shows, the chromosome seen here is V-shaped, this being caused by the median spindle fibre attachment. In the anaphase chromosome group, besides the chromosome A there are two more, which are also distinct from the others, and are to be designated as B and C respectively. Chromosome B is also V-shaped, but is much smaller than A. Chromosome C is L-shaped, owing to the sub-median fibre attachment.

The remaining five chromosomes have the subterminal fibre attachment, and are almost equal in size and shape. The chromosomes A, B, and C may be identified also in the polar view of the anaphase of the same mitosis (Fig. 3).

Text-fig. 4, a is the polar view of the metaphase of the homotypic mitosis. The chromosomes A, B and C can be detected here without any difficulty, as also in the anaphase stage of this division (Fig. 4, b).

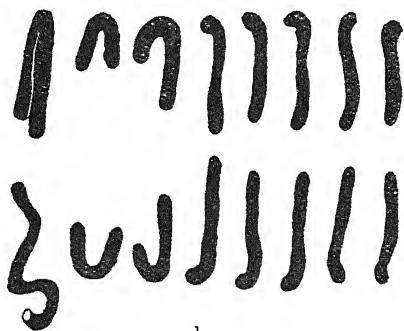
The first nuclear division in the pollen grains comes about a week after the maturation division in the pollen mother cells. The metaphase of that division is well suited for



Fig. 3.—Polar view of anaphase chromosome group of 1st division in pollen mother cells.  $\times 1760$ .



a



b

Fig. 4.—a. Polar view of metaphase of 2nd division in pollen mother cell. b. Large chromosomes in anaphase of the same division.  $\times 1760$ .

the study of chromosome morphology, as LEVAN specially mentions in his recent paper on *Allium* (1932). Text-fig. 5, a and b are the polar views of the nuclear plate of this division. Eighteen chromosomes are counted as before. Among them eight are much larger than the rest. The chromosomes A, B and C are distinguishable with certainty. The constriction for fibre attachment is here rather distinct in each chromosome and

becomes more conspicuous in the anaphase stage (Fig. 6).

The diploid somatic division was studied in the root tip. The constriction of the chromosome is distinct also in this division. Text-fig. 7 is a polar view of the nuclear plate of this division. Thirty-six chromosomes

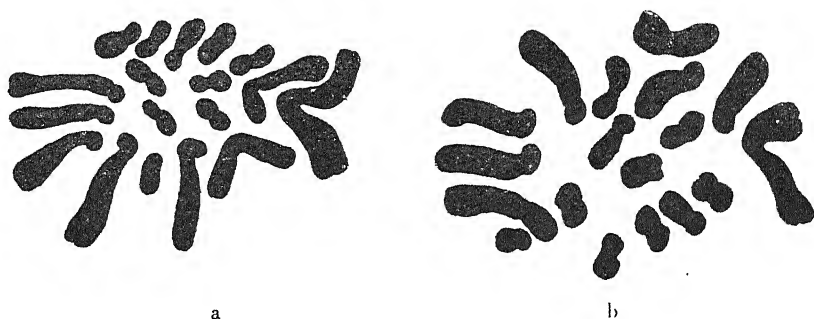


Fig. 5, a, b.—Polar views of metaphase in 1st division in pollen grain.  $\times 1760$ .

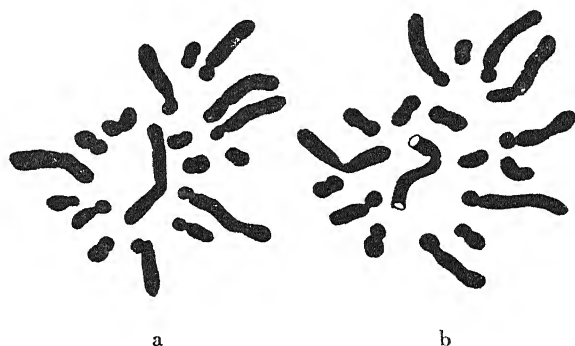


Fig. 6, a, b.—Sister chromosome group in anaphase of 1st division in pollen grain.  $\times 1760$ .

are counted; among them the chromosomes A, B and C can be detected as before. To my astonishment, a few figures of the tetraploid nuclear division in the cortex of a root, one example of which is depicted in text-fig. 8, were, by chance, met with in one preparation. The size of the nucleus of the cells in the neighbourhood of the tetraploid mitotic figures is generally much larger than the normal diploid nucleus. It is, therefore, likely that not a single cell but a relatively large portion of the root is equipped with the tetraploid nucleus. The same phenomenon has already been reported in the case of several plants (LESLEY, 1925; NAWASCHIN, 1926; BRESLAWETZ, 1926; TAKAGI, 1928).



Fig. 7.—Diploid nuclear plate in a root tip.  $\times 1760$ .



Fig. 8.—Tetraploid nuclear plate in cortex of a root tip.  $\times 1760$ .

#### SUMMARY.

1. The haploid chromosome number of *Smilacina japonica* is eighteen.
2. Among the eighteen, eight are much larger than the remaining ten. The former are situated generally in the periphery of the nuclear plate.
3. In the anaphase of the 1st and 2nd nuclear division in the pollen mother cells three sets of special chromosomes can be distinguished with certainty.
4. The first division in the pollen grain is very suitable for the study of chromosome morphology. The three special chromosomes above-mentioned can be distinguished most easily in this division.
5. The diploid nuclear mitosis was studied in the root tips. On one occasion a case was met with, where a relatively large portion of a root consisted of cells with a tetraploid nucleus.

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# IS THE PROTEIN REQUIREMENT IN NUTRITION AN AMINO ACID OR A PEPTIDE REQUIREMENT?

By

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(Received Dec. 1, 1932)

Ever since it was first emphasized by LIEBIG 100 years ago, the protein requirement has been considered the chief requirement in nutrition. LIEBIG attempted to make a concentrated food by autoclaving beef-muscle and separated it into a white powder (protein) and a brown liquid (extract of beef). Owing to the fact that the protein is tasteless, the public eats the extract and is ignorant of the existence of the protein. KOSSEL suggested that protein is a polypeptide of amino acids and this view has many supporters. IKEDA showed that the chief flavoring substance in "protein" food is the monosodium salt of glutamic acid (an amino acid). It thus appears that although the ordinary man cannot tell whether he is eating pure protein or pure starch (carbohydrate), he can detect an amino acid that is abundant in both animal and plant protein; and thus a longing for the "flesh-pots of Egypt" may be satisfied by the hydrolysis of plant protein. The taste of the free amino acids has usually been described as sweet or slightly bitter or sour (acid) but most commercial preparations of proline have a strong taste or smell, probably due to decomposition products.

Since it has been shown that some free amino acids are produced in the digestion of protein and that natural peptides may be split into amino acids by the erepsin of the small intestinal juice, the idea is prevalent that a mixture of amino acids could be substituted for protein in nutrition. Early attempts at solving this question were beset by numerous difficulties. The mixtures of amino acids were sometimes nauseating. The experiments were begun before the discovery of vitamins which were therefore not supplied. The expense of the experiments limited their duration.

ABDERHALDEN showed that yeast and butter-fat improved the diet of amino acids, attributing their beneficial effect to vitamins. The yeast contains protein but not nearly enough for the entire protein requirement.



ROSE showed that young white rats would grow on a diet containing 2 decigrams of dry yeast per day and in which the remainder of the protein requirement was a mixture of known amino acids plus a butyl alcohol extract of a casein hydrolysate. We do not wish to compete with ROSE's expensive experiments but they naturally give rise to speculations as to the nature of the butyl-alcohol extract. Using the whole of the casein hydrolysate, its effect is diminished by prolonged hydrolysis, and this might be due to destruction of traces of peptides.<sup>1)</sup>

Insulin seems to be a peptide and although most of it is destroyed in the digestive tract, MURLIN has shown that some may be absorbed before destruction. The active agent in liver which relieves the symptoms of pernicious anemia has been thought to be a peptide. A gastric digest of muscle may be substituted for liver and this gastric digest is a mixture of a large number of peptides (peptone). The quantity of liver needed to relieve pernicious anemia is enormous and this would suggest partial destruction of the active agent in the digestive tract.

Dogs can be kept alive on a certain diet after removal of the stomach, but so can they after removal of the parathyroid glands. The stomach is removed from humans only in cases of cancer or other serious disease. The patients have not lived more than 6 years and most of them have died much earlier, some within a few weeks, even where the operation did not kill them. In cases where a blood examination was made, anemia has been found. This is not intended to be an adequate discussion of the function of the stomach but is introduced as the suggestion which led us to the experiments on feeding peptides.

Owing to the great expense of the experiments we have used mice and only one pair of litter-mate males for each experiment. We have given them an adequate mineral mixture and sufficient fat and vitamins A and D in the form of butter-fat. We have given each the equivalent of 1 decigram of dry yeast per day but have not shown that this supplied sufficient vitamin B and G. Owing to the fact that yeast contains protein, however, we have not dared to increase it. We have in each experiment used 2 diets which differed only in that one contained peptides and the other did not. We have used only those peptides which we had in a pure state. The ingredients of the diets were weighed in decigrams.

Diet I contained glycine 1, proline 4, hydroxyproline 1, aspartic acid 10, glutamic acid 10, serine 1, alanine 4, valine 8, leucine 10, isoleucine

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<sup>1)</sup> McCLENDON: Proc. Soc. Exp. Biol. Med. 28:915 (1931).

1, phenylalanine 3, tyrosine 5, arginine 3, lysine 4, histidine 2, tryptophan 1, cystine 1, methionine 1, taurine 1, creatine 1, adenine 1, guanine 1, hemin 1, glucosamine 1, cholesterol 1, glucose 75, starch 150, salt-mixture 20 (butter-fat 80). The yeast was fed separately, 1 decigram per mouse per day. This diet (omitting the butter fat) was mixed by grinding in a pyrex ball mill for 6 hours then divided into halves.

Diet II consisted of 1/2 of diet I plus 1 decigram each of the following: glycyl-glycine, glycine-anhydride, glycyl-leucine, leucyl-glycine, glycyl-tyrosine, tri-glycine, leucyl-diglycine, glutathione and alanyl-triglycine. This was ground again in the ball mill. The butter-fat was halved and half mixed in diet I and half in diet II in small portions as needed each day.

The mice were the Bagg albinos from the Carnegie Station for Experimental Evolution, Cold Spring Harbor, Long Island, N.Y., U.S.A., which are the standard strain for uniform scientific work since they were inbred for many years and are therefore homozygous. The body weights are given in decigrams.

#### Experiment 1 on litter-mate males born October 1:

Date	Oct.	22	23	24	25	26	27	28	29	30	31
Body	Diet I	50	53	52	55	53	50	49	48	49	48
Weight	Diet II	50	57	55	55	55	51	56	51	50	49

Date	Nov.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Body	Diet I	46	46	46	47	47	48	48	46	45	44	44	43	40	40	40	40 dead
Weight	Diet II	50	49	49	48	48	47	48	48	47	47	47	45	43	44	43	43 alive

#### Experiment 2, litter-mate males born September 2:

Date	Nov.	18	19	20	21	22	23	24	25	26	27
Body	Diet I	165	160	151	141	137	133	130	128	127	125
Weight	Diet II	142	135	129	123	119	120	114	111	113	110

#### Experiment 3, litter-mate males born August 18:

Date	Nov.	28	29	30	Dec.	1	2	3	4	5	6	7
Body	Diet I	213	199	191		191	182	180	177	166	160	156
Weight	Diet II	195	190	180		170	165	159	156	149	145	140

It may be concluded that the results are negative. Younger (smaller) mice do better on both diets than do older (larger) mice. This may be due to the well known fact that younger animals adapt themselves to

food of abnormal taste more quickly than older animals or to the possibility that smaller animals require less yeast than larger animals.

Our thanks are due to Dr. E. WALDSCHMIDT-LEITZ for some of the peptides.

After writing this paper we read of the experiments of MAEDA<sup>1)</sup> in which he obtained normal growth in rats fed protein hydrolysates instead of protein. He admits detecting amino acid anhydrides (which we assume are diketopiperazines rather than creatinine) in some of the hydrolysates. The problem is open to further investigation.

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<sup>1)</sup> MAEDA, S.: Sci. Papers Ins. Phys. Chem. Research, Tokyo 19:67 (1932).

# NOTES ON THE ANATOMY OF THE YOUNG OF *CAUDINA CHILENSIS* (J. MÜLLER).<sup>1)</sup>

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(With 31 text-figures and Pls. II-III).

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It seems that no particular investigation has been made of the morphology of the young of the holothurians, which belong to the Molpadiidae, and it is probable that this omission is due to the difficulties in obtaining suitable material.

Fortunately in the waters about the Asamushi Marine Biological Station we find a kind of holothurian, *Caudina chilensis* (J. MÜLLER), which belongs to the above-mentioned family and not only the adult but also the young forms and larvae of various stages are obtainable in great abundance, according to the season of the year. In the present paper I should like to deal with the results of investigation of the anatomy of the young *Caudina*.

Here I wish to express my hearty thanks to Professor S. HATAI, at whose suggestion this work was undertaken and I am also grateful to Professor S. HÔZAWA for kind help given to me in various ways.

## MATERIAL AND METHOD.

The specimens of the young *Caudina* on which the present work is based were of varying body-length, measuring 1-30 mm. and were obtained by Prof. S. HÔZAWA and by myself near the Biological Station of Asamushi at Moura and Nonai during the breeding season. Each of the animals was first anaethetised with menthol, and then fixed with 90% alcohol. In the preserving materials, 70% of alcohol was used.

The material of the cut sections was totally stained with borax carmine, and was then imbedded in either paraffin or celloidin. The celloidin method was well adapted for the larger specimens.

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<sup>1)</sup>Contributions from the Marine Biological Station, Asamushi. Aomori-Ken. No. 98.

## ABBREVIATIONS FOR ALL FIGURES IN THE TEXT AND PLATES.

<i>a.</i> , anus	<i>m.</i> , mouth
<i>a.e.v.</i> , anterior efferent vessel	<i>md.</i> , madreporite
<i>a.p.</i> , anal papilla	<i>m.f.</i> , muscular fibres
<i>a.s.i.</i> , anterior small intestine	<i>m.v.r.w.c.</i> , mid-ventral radial water canal
<i>a.v.</i> , afferent vessel	<i>n.r.</i> , nerve ring
<i>b.c.</i> , body cavity	<i>o.b.</i> , outer band
<i>b.t.</i> , basal tree	<i>o.e.</i> , outer epithelium
<i>b.w.</i> , body wall	<i>p'</i> , primary papilla
<i>cap.</i> , capillaries	<i>p''</i> , secondary papilla
<i>c.b.</i> , calcareous body	<i>p'''</i> , tertiary papilla
<i>cl.</i> , cloaca	<i>p.d.</i> , primary digit
<i>cm.</i> , circular muscle	<i>p.e.v.</i> , posterior efferent vessel
<i>com.</i> , commissure	<i>ph.</i> , pharynx
<i>c.p.c.</i> , coelo-peripharyngeal connection	<i>p.s.</i> , peri-pharyngeal sinus
<i>c.p.s.</i> , coelo-peripharyngeal septum	<i>p.s.i.</i> , posterior small intestine
<i>cr.</i> , constriction	<i>ps.s.</i> , peri-stomachal sinus
<i>c.r.</i> , calcareous ring	<i>P.v.</i> , Polian vesicle
<i>ct.p.</i> , connective tissue partition	<i>r.cl.m.</i> , radial cloacal muscle
<i>cut.</i> , cuticle	<i>r.d.m.</i> , right dorsal mesentery
<i>c.w.c.</i> , circular water canal	<i>r.d.r.w.c.</i> , right dorsal radial water canal
<i>db.v.</i> , diagonal blood vessel	<i>r.n.</i> , radial nerve
<i>e.c.</i> , epi-neural canal	<i>r.r.t.</i> , right respiratory tree
<i>e.v.</i> , efferent vessel	<i>r.s.r.t.</i> , right supernumerary respiratory tree
<i>g.</i> , gonad	<i>r.v.m.</i> , right ventral mesentery
<i>g.d.</i> , gono-duct	<i>r.v.r.w.c.</i> , right ventral radial water canal
<i>h.c.</i> , hypo-neural canal	<i>r.v.t.</i> , right ventral tree
<i>i.b.</i> , inner band	<i>r.w.c.</i> , radial water canal
<i>i.e.</i> , inner epithelium	<i>s.d.</i> , secondary digit
<i>l.c.t.</i> , layer of connective tissue	<i>sp.</i> , suspensor
<i>l.d.b.</i> , left dorsal branch	<i>st.</i> , stomach
<i>l.d.m.</i> , left dorsal mesentery	<i>st.c.</i> , stone canal
<i>l.d.r.w.c.</i> , left dorsal radial water canal	<i>t.</i> , tentacle
<i>l.i.</i> , large intestine	<i>t'</i> , primary tentacle
<i>l.m.</i> , longitudinal muscle	<i>t''</i> , secondary tentacle
<i>l.r.t.</i> , left respiratory tree	<i>t'''</i> , tertiary tentacle
<i>l.s.r.t.</i> , left supernumerary respiratory tree	<i>t.a.</i> , tentacular ampulla
<i>l.v.b.</i> , left ventral branch	<i>v.t.</i> , ventral tree
<i>l.v.m.</i> , left ventral mesentery	<i>v.v.</i> , ventral vessel
<i>l.v.r.w.c.</i> , left ventral radial water canal	<i>w.ps.s.</i> , wall of peri-stomachal sinus
<i>l.v.t.</i> , left ventral tree	

## ANATOMY OF THE YOUNG.

In describing the anatomy of the young *Caudina*, it seems to be more convenient to distinguish and mark off the various stages of growth. I

have divided, therefore, these specimens into four stages distinguished by the number and shape of the tentacles as well as by the length of body, as shown in the following table. At the end of the fourth stage the animal becomes almost similar to the adult condition.

TABLE I.

Stages	I	II	III	IV
Body length	1-2 mm.	2-4 mm.	4-10 mm.	10-30 mm.
Number of tentacles	6-10	11-15	15	15
Shape of tentacles	With two digits.	" "	With four digits, two secondary digits being smaller ones.	With four digits of similar appearance.

## I. THE FIRST STAGE

In this stage the animal measures about 1-2 mm. in total length and is almost spindle-shaped, but the tail is not yet strongly elongated as it is in the adult (text-fig. 1). The integument is translucent, but is covered with sand grains attached all over its surface.

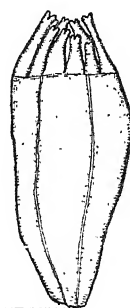
### 1. *Body Wall*

The body wall consists of four layers: an outer epithelium, a layer of connective tissue, a muscular layer, and a thin inner epithelium, as in the case of the adult.

The outer epithelium (Pl. II, fig. 1, *o.e.*) is thin, and is covered with a very thin cuticle (*cut.*).

The layer of connective tissue (*l.c.t.*) is thick, and the muscular fibres are very thinly distributed in it. Calcareous bodies are observable in this layer but only in the anal region.

The five longitudinal muscles (*l.m.*) exist, each in the position of a radius, and each forming a single strand. They arise at the bases of the tentacles together with the radial nerves, and run posteriorly to the anal region. The circular muscles (*c.m.*) lie in the inter-radial areas, stretched



Text-fig. 1. *Caudina chilensis* (J. MÜLLER). The young 1.8 mm. in body length.

transversely among the longitudinal muscles, but they do not form any continuous sheet.

## 2. Nervous System

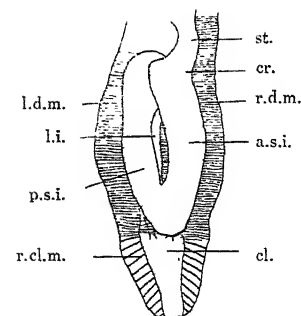
The nervous system consists chiefly of the nerve ring and the five radial nerves.

The nerve ring (Pl. II, fig. 2, *n.r.*) is seen at the base of the tentacles surrounding the mouth and is about 0.15 mm. in diameter.

The five radial nerves (figs. 1, 2, *r.n.*), which branch off from the nerve ring, stretch towards the posterior extremity lying between the epi- and hypo-neural canals (fig. 1, *e.c.*, *h.c.*).

## 3. Digestive System

The alimentary canal is looped in the same way as seen in the adult, namely, it runs first towards the posterior end of the trunk, then bends and runs forwards almost to the posterior end of the stomach, then turns again towards the tail, and finally terminates at the anus (text-fig. 2).



Text-fig. 2. *Caudina chilensis* (J. MÜLLER). Alimentary canal of the young in the first stage.

The mouth (Pl. II, fig. 2, *m.*) is circular, and is situated at the anterior end of the body.

The pharynx (fig. 3, *ph.*), which extends from the mouth to the level of the circular water canal is fastened to the calcareous ring by means of ten coelo-peripharyngeal septa (figs. 3, 6, *c.p.s.*) placed vertically and radially round the pharynx. There exist five peripharyngeal sinuses (fig. 3, *p.s.*) each of which occupies very narrow space surrounded by two coelo-peripharyngeal septa, by a part of the pharyngeal wall, and by the radial piece of calcareous ring. The posterior end of each coelo-peripharyngeal septum is connected with the wall of the peri-stomachal sinus (fig. 6, *w.p.s.*). In this stage the wall of the peri-stomachal sinus is set close to that of the stomach, not leaving the cavity for the peri-stomachal sinus. The anterior end of each septum is set freely in the coelo-peripharyngeal connection (fig. 6, *c.p.c.*) as seen in the adult.

The stomach (Pl. II, fig. 5, text-fig. 2, *st.*) is situated next to the pharynx, and is of uniform thickness. It is supported by the right dorsal mesentery (*r.d.m.*). Posteriorly, it is continuous with the small intestine and the limit between them is marked by a distinct constriction (text-fig. 2, *cr.*).

In the region of the small intestine the alimentary canal increases in diameter, and is curved in the manner of figure U (text-fig. 2). The first half of the small intestine (anterior small intestine) (Pl. II, fig. 4, text-fig. 2, *a.s.i.*) is supported by the right dorsal mesentery (Pl. II, fig. 4, text-fig. 4, *r.d.m.*), while the second half (posterior small intestine) (Pl. II, fig. 4, text-fig. 2, *p.s.i.*) is supported by the left dorsal mesentery (Pl. II, fig. 4, text-fig. 2, *l.d.m.*).

The large intestine (Pl. II, fig. 4, text-figs. 2, 3, *l.i.*) which runs posteriorly and ventrally to the small intestine is supported by the left and right ventral mesenteries (*l.v.m.*, *r.v.m.*).

The cloaca (text-fig. 2, *cl.*), which is very short but comparatively thick, is dilated in the anterior region, where the respiratory trees arise, and terminates posteriorly in the anus. It is supported by the radial cloacal muscles (*r.cl.m.*).

#### 4. Respiratory Trees

In the region of the cloaca two respiratory trees arise from its dorsal side and run forwards. Of these trees, the right (Pl. II, fig. 4, text-fig. 3, *r.r.t.*) reaches anteriorly to the circular water canal, which will be mentioned later on, and gives off no branches on its way, while the left is divided near its base into two branches, viz. the bud-like left dorsal branch (text-fig. 3, *l.d.b.*) and the club-shaped left ventral branch (*l.v.b.*).

The supernumerary respiratory trees which are demonstrable in the later stages have not yet appeared in this stage.

#### 5. Calcareous Ring

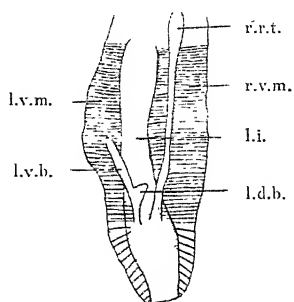
The calcareous ring (Pl. II, figs. 3, 6, *c.r.*) established in this stage as to surround the pharynx has a diameter of about 0.2 mm.

#### 6. Water-vascular System

The circular water canal (Pl. II, fig. 6, text-fig. 4, *c.w.c.*) is to be seen surrounding the junction of the pharynx and the stomach.

The short stone canal (Pl. II, fig. 5, text-fig. 4, *st.c.*) is noticeable having its position in the median dorsal inter-radius, and is nearly straight and not twisted as it is in the later stages.

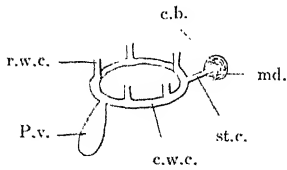
The madreporite (*md.*) is circular in shape and is studded with calcareous bodies (*c.b.*). It is attached to the body wall in this stage.



Text-fig. 3. *Caudina chilensis* (J. MÜLLER). The general view of the respiratory trees of the young in the second stage.



The Polian vesicle (Pl. II, fig. 5, text-fig. 4, *P.v.*) is a comparatively large sac of elongated oval shape and its position is in the left ventral interradius.

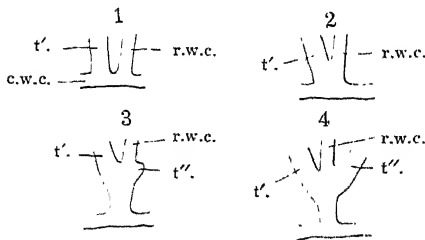


Text-fig. 4. *Caudina chilensis* (J. MÜLLER). Diagrammatic illustration of the water-vascular system of the young in the first stage.

The radial water canals (Pl. II, fig. 1, 3, text-fig. 4, *r.w.c.*), which are five in number, arise from the circular water canal, and each runs backwards between the radial nerve and the longitudinal muscle reaching as far as the posterior extremity of the body.

The primary tentacles (Pl. II, fig. 2, text-figs. 5, 6, *t'*.) which are five in number, appear early in the larval stage, arising from the circular water canal at the bases of the radial water canals as stated by INABA (1930, pp. 232-233). But, afterwards, as the animal is growing the basal part of the radial water canal is prolonged, and then the tentacles look like branches ramifying from the radial water canals (text-fig. 5).

The secondary tentacles (*t''*.) which arise in this stage are also five in number, and they are given off from the radial water canals. Their positions are as follows: the first (text-



Text-fig. 5. *Caudina chilensis* (J. MÜLLER). Diagrammatic illustration for the formation of the primary tentacles of the young in the first stage. The number of the figure shows the order of the development of the tentacles.

fig. 6, *t''*<sub>1</sub>.) is right dorsal coming out from the right ventral radial water canal (*r.v.r.w.c.*); the second (*t''*<sub>2</sub>) is left dorsal arising from the left ventral radial water canal (*l.v.r.w.c.*); the third (*t''*<sub>3</sub>.) is dorsal arising from the right dorsal radial water canal (*r.d.r.w.c.*), and each of the fourth and the fifth (*t''*<sub>4,5</sub>.) is either left ventral or right ventral arising from the left ventral radial water canal, or from the right ventral.

At the end of this stage the tentacles are ten in all, and each is provided with two primary digits at its tip (text-fig. 7, *p.d.*). The tentacular ampullae are not yet formed in this stage.

The anal papillae (text-fig. 1, 8, *a.p.*, *p'*.) each of which is to be found at the posterior end of the radial water canal appear first as a single primary protuberance in this stage.

### 7. Blood-vascular System

In this stage the dorsal blood vessel is represented only by the efferent vessel which exists on the dorsal surface of the posterior small intestine. (Pl. II, fig. 4, text-fig. 9, *e.v.*).

In the early period of this stage the ventral blood vessel may be found running along the ventral surface of the anterior small intestine (*v.v.*), but, in the more advanced period of the same, it may be seen ventrally even on the posterior small intestine (text-fig. 10, *v.v.*).

### 8. Genital Organ

The genital organ is not yet clearly distinguishable in this stage.

## II. THE SECOND STAGE

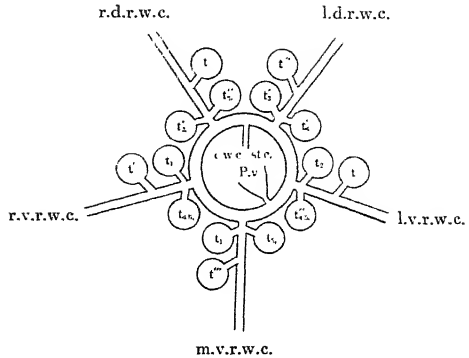
In this stage the total length of the body varies from about 2 to 4 mm. In respect to the shape of the body it is almost similar to that of the previous stage, the tail being very short. As is the case in the first stage the body is covered with sand grains nearly all over the surface.

### 1. Body Wall

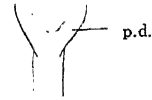
In this stage the layer of connective tissue is thicker than in the former stage, and the muscular fibres contained in that layer are increased.

The calcareous bodies first appear in the posterior region of the body, and later they are found not only in that region but also in the anterior parts of the body.

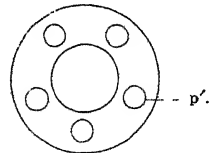
Each of the longitudinal muscles shows a tendency to divide into two longitudinal bands, and consequently, the cross-section of the muscle is nearly elliptical showing a slight constriction in the middle.



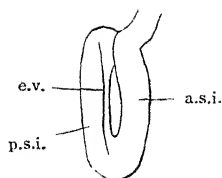
Text-fig. 6. *Caudina chilensis* (J. MÜLLER). Diagram to show the arrangement of the tentacles of the young in the first stage. The number shows the order of appearing of tentacle.



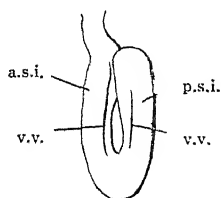
Text-fig. 7. *Caudina chilensis* (J. MÜLLER). A tentacle with two digits at its tip of the young in the first stage.



Text-fig. 8. *Caudina chilensis* (J. MÜLLER). Diagram showing the arrangement of the anal papillae of the young in the first stage.



Text-fig. 9. *Caudina chilensis* (J. MÜLLER). Diagram to show the dorsal blood vessel of the young in the first stage.



Text-fig. 10. *Caudina chilensis* (J. MÜLLER). Diagram showing the position of the ventral blood vessel.

## 2. Nervous System

The nerve ring becomes larger, increasing in diameter. It measures about 0.2 mm. in this stage.

## 3. Digestive System

The general features of the alimentary canal in this stage are nearly similar to those seen in the previous stage.

At the end of this stage the wall of the peri-stomachal sinus is separated from that of the stomach, and the peri-stomachal sinus (Pl. II, figs. 5, 6, *ps.s.*) is thus formed. The suspensors (*sp.*) also become observable, and they connect both walls mentioned above.

## 4. Respiratory Trees

In this stage the right respiratory tree is divided into two branches at its terminal portion (Pl. II, fig. 5, *r.r.t.*) while the left is more elongated than in the first stage.

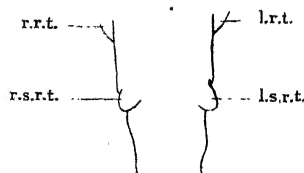
The supernumerary respiratory trees (text-figs. 11, 12, 13, *l.s.r.t.*, *r.s.r.t.*) appear first in this stage as two bud-like processes each arising from the base of the main tree. Each of these buds is divided into two branches late in this stage.

## 5. Calcareous Ring

The calcareous ring in this stage is larger than that in the previous one and its diameter measures about 0.3 mm.

## 6. Water-vascular System

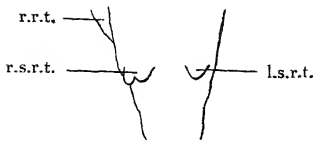
The stone canal (Pl. II, fig. 5, text-fig. 14, *st.c.*) is not yet twisted, but the wall is studded with calcareous bodies in the region close to the madreporite.



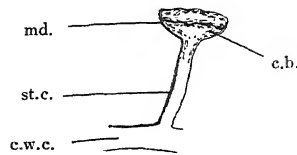
Text-fig. 11. *Caudina chilensis* (J. MÜLLER). The ventral view of the cloaca of the young about 3 mm. long.



Text-fig. 12. *Caudina chilensis* (J. MÜLLER). The right side view of the same as text-fig. 11.



Text-fig. 13. *Caudina chilensis* (J. MÜLLER). The ventral view of the cloaca of the young about 3.3 mm. long.



Text-fig. 14. *Caudina chilensis* (J. MÜLLER). The stone canal and the madreporite of the young in the second stage.

The madreporite (*md.*) is attached to the body wall, and its margin shows some undulation.

In this stage five tertiary tentacles (text-fig. 6, *t'''*.) are formed: one is right dorsal in position arising from the right ventral radial water canal (*r.v.r.w.c.*); one is right ventral arising from the mid-ventral radial water canal (*m.v.r.w.c.*); one is left dorsal arising from the left ventral radial water canal (*l.v.r.w.c.*), and the remaining two are mid-dorsal each arising from either the right or left dorsal radial water canal (*r.d.r.w.c.*, *l.d.r.w.c.*). Thus the animal in this stage has fifteen tentacles, and as regards their position it may be said that four are mid-dorsal, three right dorsal, three right ventral, three left dorsal, and the remaining two left ventral. Each of these tentacles has two digits at its tip, as in the case of the previous stage (text-fig. 7). The rudimentary tentacular ampullae (Pl. II. fig. 6, *t.a.*) are first formed at the bases of the primary and of the secondary tentacles in this stage.

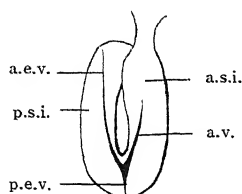
In the early period of this stage each of the primary anal papillae is represented only by a single protuberance, but later on at the end of this stage, more smaller secondary protuberances (text-fig. 22, *p''*.) appear in addition to each of the primary ones.

#### 7. *Blood-vascular System*

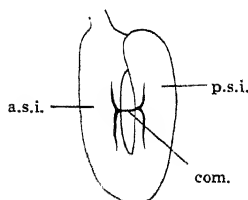
The afferent branch of the dorsal blood vessel and the commissures between two ventral vessels are developed in this stage. Thus the dorsal blood vessel is differentiated into the efferent and the afferent branches, and the ventral blood vessel is provided with one or two commissures.

The afferent vessel (text-fig. 15, *a.v.*) which appears on the dorsal surface of the anterior small intestine runs posteriorly being attached to the wall of the same. Near the posterior intestinal bending, it is divided into two branches, the anterior efferent vessel and the posterior efferent. The anterior efferent vessel (*a.e.v.*) runs anteriorly along the dorsal surface

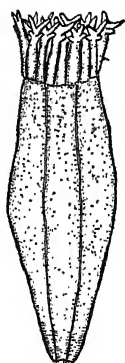
of the posterior small intestine, while the posterior efferent vessel (*p.e.v.*) goes posteriorly for a short distance, across the surface of the small intestine.



Text-fig. 15. *Caudina chilensis* (J. MÜLLER). The dorsal blood vessel of the young in the second stage.



Text-fig. 16. *Caudina chilensis* (J. MÜLLER). The ventral blood vessel of the young in the second stage.



Text-fig. 17. *Caudina chilensis* (J. MÜLLER). The young in the third stage with a body length of about 9 mm.

The vessels which run longitudinally along the antero-posterior ventral part of the small intestine, are connected by one or two transverse commissures (text-fig. 16, *com.*).

### 8. Genital Organ

The genital organ is not yet differentiated in this stage.

## III. THE THIRD STAGE

In this stage the total length of the animal measures about 4–10 mm. and at the end of this stage the tail is more or less elongated (text-fig. 17). Sand grains cover the body surface all over as is the case in the previous stage.

### 1. Body Wall

In this stage the layer of connective tissue (Pl. II, fig. 7, *l.c.t.*) is thicker than in the last stage. Calcareous bodies (*c.b.*) are very thinly scattered in the anterior portion of the body, but they are thickly disposed towards the anal region, as stated by HÔZAWA (1928, p. 363).

Each of the longitudinal muscles (*l.m.*) increases in breadth and is more clearly divided into a pair than in the previous stage. The separation into a pair of the longitudinal muscles occurs only in its middle parts, and thus both ends remain in the form of a single strand.

### 2. Nervous System

The nerve ring increases in thickness as well as in diameter, the latter measuring about 0.4–0.8 mm.

The radial nerve (Pl. II, fig. 7, *r.n.*) which is crescentic in the cross-section, is more thickened and more elongated than in the previous stage. In structure, the following three components may be clearly distinguished, viz.

a thick outer band (*o.b.*), a thin connective tissue partition (*c.t.p.*), and a thin inner band (*i.b.*).

### 3. Digestive System

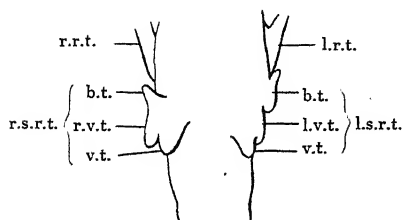
In this stage each digestive canal is enlarged, but the cloaca is not strongly prolonged.

### 4. Respiratory Trees

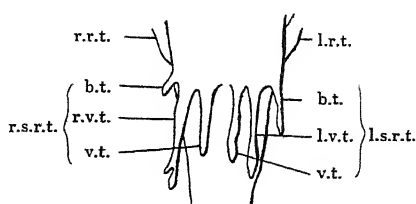
In this stage, many side-branches of the respiratory trees are formed, each arising from the latter in a bud-like form.

The anterior end of the right respiratory tree reaches the level of the calcareous ring. In this stage the left dorsal branch of the left respiratory tree is divided into a small number of smaller branches near its base, but these are not mingled with the capillaries, which spread from the dorsal afferent vessel.

In this stage each of the two supernumerary respiratory trees, which first appeared in the last stage as two bud-like protuberances from the ventral surface of the cloaca, at a level slightly posterior to that of the main respiratory trees, is now divided into three branches (text-fig. 18, 19, *l.s.r.t.*, *r.s.r.t.*).



Text-fig. 18. *Caudina chilensis* (J. MÜLLER). The ventral view of the cloaca of the young about 5 mm. long.



Text-fig. 19. *Caudina chilensis* (J. MÜLLER). The same of the young as text-fig. 18, about 6 mm. long.

### 5. Calcareous Ring

In the present stage the calcareous ring has grown larger than in the last, and attains 0.3–1.1 mm. in diameter.

### 6. Water-vascular System

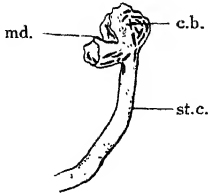
In this stage the stone canal (text-fig. 20, *st.c.*) is more or less elongated and somewhat twisted.

Further, the madreporite (*md.*) is more strongly undulated at the margin and becomes free from the body wall.

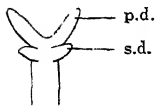
In this stage the tentacles are fifteen in number, being furnished with

two secondary digits (text-fig. 21, *s.d.*) which appear at the base of each primary digit.

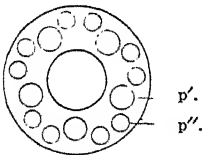
The tentacular ampullae are clearly observable, each being formed at the base of each tertiary tentacle this showing that they are fifteen in number.



Text-fig. 20. *Caudina chilensis* (J. MÜLLER). The stone canal and the madreporite of the young in the third stage.



Text-fig. 21. *Caudina chilensis* (J. MÜLLER). The tip of the tentacle of the young in the third stage.



Text-fig. 22. *Caudina chilensis* (J. MÜLLER). Diagrammatic illustration of the arrangement of the anal papillae of the young in the third stage.

Regarding the anal papillae, it may be said that they are set in five groups, each consisting of three papillae; a large primary one (text-fig. 22, *p'*) in the middle, and two secondary ones (*p''*) outside the primary one.

#### 7. Blood-vascular System

In this stage the blood capillaries and the diagonal blood vessel appear, the former forming the rete-mirabile which is found on the surface of the anterior small intestine. Thus the dorsal blood vessel consists of the afferent vessel, the capillaries, and the efferent vessel, while of the ventral blood vessel the commissures and the diagonal blood vessel which first appears in this stage, are distinguishable.

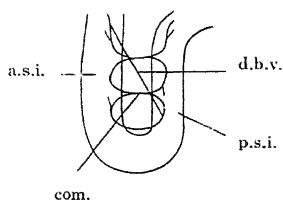
The capillaries (Pl. III, fig. 8, *cap.*) which first appear in this stage form the rete-mirabile covering the dorsal surface of the small intestine, but they do not intermingle with the twigs of the left dorsal branch of the left respiratory tree, as will be seen in the case of the later stages.

The diagonal blood vessel (text-fig. 23, *d.b.v.*) which also first appears in this stage, connects the ventral vessels, running along the anterior and the posterior small intestine diagonally.

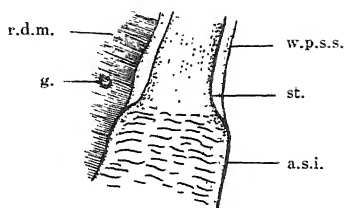
The number of the commissures which connect the ventral vessels varies from 2 to 4 in this stage (*com.*).

#### 8. Genital Organ

The gonad (Pl. III, fig. 9, text-fig. 24, *g.*) is first distinguished in this stage, being located between two layers of the right dorsal mesentery near the posterior portion of the stomach, and it is a small rounded body.



Text-fig. 23. *Caudina chilensis* (J. MÜLLER). The ventral blood vessel of the young in the third stage.



Text-fig. 24. *Caudina chilensis* (J. MÜLLER). Diagram to show the position of the gonad.

#### IV. THE FOURTH STAGE

The total length of the animal in this stage is about 10–30 mm. In the middle of this stage the tail is much elongated, and the shape of the entire animal becomes almost similar to that of the adult (text-fig. 25). Furthermore sand grains cover only the posterior portion of the body.

##### 1. Body Wall

In the region of the body surface, where it is not covered by sand grains, the outer epithelium (Pl. III, fig. 10, *o.e.*) is very smooth.

The muscular fibres (*m.f.*) contained in the connective tissue layer are well-developed in this stage.

The calcareous bodies (*c.b.*) are thinly scattered throughout the integument of the trunk, but are found abundantly in the tail region.

The longitudinal muscles (*l.m.*) are set in five radial groups, each of them consisting of two muscle bands. The muscle bands which make a pair are of equal width in the middle region of the body, but combine to form one piece at both extremities.

##### 2. Nervous System

The nerve ring attains a diameter of 0.8–1.1 mm.

##### 3. Digestive System

In the stage, the cloaca is strongly elongated relatively to the growth of the tail.



Text-fig. 25. *Caudina chilensis* (J. MÜLLER). The young in the fourth stage with a body length of about 20 mm.



#### 4. Respiratory Trees

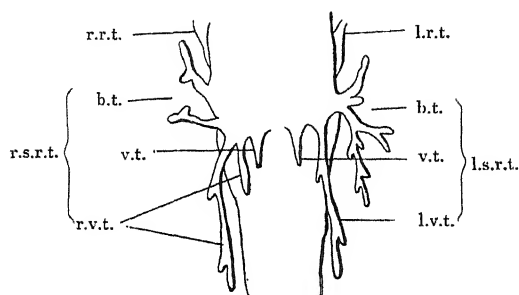
The side-branches of the respiratory trees increase in number.

The right respiratory tree reaches as far as the level of the anterior extremity of the coelom. The anterior extremity of the left ventral branch of the left respiratory tree attains the level of the anterior bending of the intestine, while the left dorsal branch of the same is divided into many smaller branchlets, which are joined to the rete-mirabile formed by

the blood capillaries belonging to the dorsal afferent vessel (Pl. III, fig. 12).

The supernumerary respiratory trees (text-fig. 26, *l.s.r.t.*, *r.s.r.t.*) grow larger, and their side-branches increase in number.

I shall now deal with these trees more in detail. The supernumerary respiratory trees (Überzählige Kiemenbäume by LUDWIG 1889-92, pp. 169-171) have, hitherto only been observed in the families Cucumariidae and Molpadiidae.



Text-fig. 26. *Caudina chilensis* (J. MÜLLER). The ventral view of the cloaca of the young about 13 mm. long.

As regards the Molpadiidae three respiratory trees, two normal and one supernumerary, were reported in the case of the following species; *Molpadia australis* SEMPER (SEMPER, 1868), *Haplodactyla molpadioides* SEMP. (SEMP. 1868), *H. australis* SEMP. (SEMP. 1868), *H. hyaloeides* SLUITER (SLUITER, 1880), *Trochosoma albicans* THEEL (THEEL 1886), *T. arcticum* (v. MARENZELLER) (v. MARENZ. 1877), and in *Caudina ransonetti* v. MARENZ, (v. MARENZ. 1881). Of the above-mentioned species with the exception of *C. ransonetti*, it has been known that there exists only one supernumerary respiratory tree issuing from the base of the left respiratory tree, while in the case of *C. ransonetti*, a species which may be looked upon as identical with *C. chilensis*, the existence of many basal branches of the main respiratory trees, which later were called supernumerary trees by LUDWIG (1889-92, p. 170) has been reported by v. MARENZELLER (1881, p. 127).

The existence of five respiratory trees was first reported by BRANDT (1835) in the cases of the genera *Liosoma* and *Aspidochir*, and afterwards by GRUBE (1840) in the case of genus *Haplodactyla*. SELENKA (1868) and LAMPERT (1885) also reported that there were some holothurians, which have five respiratory trees. But these observations were not confirmed by LUDWIG (1889-92, p. 171).

*Caudina chilensis* (J. MÜLLER) has six or more supernumerary respiratory trees (Pl. III, fig. 11, *l.s.r.t.*, *r.s.r.t.*) originating from two buds appearing at the base of the main respiratory tree, as already mentioned (p. 50).

The length of each supernumerary respiratory tree is shorter than that of the main tree, but in the young holothurians it becomes gradually longer

as they grow. Their length is not always said to be proportional to that of the body, *i.e.* there exist some individuals, in which the length of the supernumerary trees is more or less differentiated, though they are nearly equal in body length.

The direction taken by the supernumerary trees is, roughly speaking, as mentioned below. The basal tree which arises from the base of the main respiratory tree runs either anteriorly or posteriorly, while the remaining four run posteriorly, passing through the interspaces between the radial cloacal muscle (Table II). They are supported at their bases by the posterior portion of the ventral mesenteries and thus no change is possible in the direction they take.

Though more than six supernumerary trees may often occur, there are never less than six in the case of the present holothurian, three occurring on each side.

TABLE II.

Body length of animal	Left supernumerary trees			Right supernumerary trees		
	Basal tree	Left ventral tree	Ventral tree	Basal tree	Right ventral tree	Ventral tree
5.	0.07(f)	0.07(b)	0.2(b)	0.07(f)	0.08(b)	0.17(b)
5.	0.14(b)	0.16(b)	0.24(b)	0.1(f)	0.3(b)	0.34(b)
6.	0.14(b)	0.26(b)	0.2(b)	0.07(b)	0.26(b)	0.2(b)
8.	0.16(f)	0.32(b)	0.26(b)	0.07(b)	0.35(f)	0.2(b)
10.	0.13(f)	0.26(b)	0.2(b)	0.13(f)	0.26(b)	0.2(b)
13.	0.75(b)	0.73(b)	0.26(b)	0.82(b)	0.4(b)	0.17(b)
20.	1.36(b)	0.72(b)	0.32(b)	1.2(b)	0.64(b)	0.28(b)
30.	3(f)	3(b)	1.3(b)	2(f)	3(b)	1.2(b)
39.	(2.7(b) 1.2(f)	1.8(b)	1.7(b)	1.8(f)	2.5(b)	1.8(b)
55.	5.5(f)	6(b)	(3(b) 1.6(b) 2.7(b)	4(f)	4(f)	6(b)
82.	(12(b) 6(f)	5.5(b)	6.7(b)	7(b)	7(b)	1.5(b)
128.	4(f)	7(b)	4(b)	2(f)	4.4(b)	5(b)
155.	15(b)	9.5(b)	9(b)	7(f)	9(b)	10(b)
160.	(15(b) 6(f)	11(b)	7(b)	6(f)	16(b)	6(b)
175.	(10(b) 4.8(f)	7.5(b)	9(b)	4(b)	8(b)	8.5(b)
180.	3(b)	14(b)	(10(b) 8(b)	8(b)	9(b)	6(b)
185.	18(b)	7(b)	6(b)	4(f)	19(b)	6(b)
195.	(3(b) 4(f)	21(b)	14(b)	(5(b) 5(f)	13(b)	11(b)
195.	9(f)	20(b)	12(b)	9(f)	11(b)	7(b)
205.	(16(b) 5(f)	8(b)	11(b)	(9(b) 6(f)	(5(b) 3(b)	3(b)
213.	10(b)	14(f)	5(b)	5(f)	14(b)	5(b)

Body length of animal	Left supernumerary trees			Right supernumerary trees		
	Basal tree	Left ventral tree	Ventral tree	Basal tree	Right ventral tree	Ventral tree
215.	10(b)	6(b)	3(b)	12(b)	6(b)	4(b)
215.	5(f)	16(b)	16(b)	9(f)	9(b)	8(b)
220.	8(f)	31(b)	6(b)	6(f)	27(b)	8(b)
225.	9(f)	16(b)	21(b)	7(f)	21(b)	19(b)
245.	16(b)	12(b)	9(b)	6(f)	12(b)	9(b)
260.	(40(b) 6(f)	33(b)	7(b)	(34(b) 8(f)	24(b)	5(b)
275.	43(b)	4(f)	7(b)	(8(b) 11(f)	34(b)	(3(b) 5(b)
290.	3(f)	20(b)	15(b)	5(f)	14(b)	5(b)

In the table, the following six supernumerary trees are dealt with, the terms taken being respectively from their position.

(1) The basal tree (Pl. III, fig. 11, text-fig. 18, 19, 26, *b.t.*) is that which arises from the cloaca at the base of the main tree. (2) The left and right ventral trees (*l.v.t.*, *r.v.t.*) are those each of which arises from the ventral surface of the cloaca on the left and right side, respectively. (3) The ventral tree (*v.t.*) is that which arises from the ventral surface of the cloaca along the mid line.

The number denotes the length of the supernumerary trees, representing in mm., while the letters f and b mean "forward" and "backward" respectively, showing the direction they take.

GEROULD did not mention these supernumerary trees in his admirable work entitled "Anatomy and Histology of *Caudina arenata* GOULD" (1896, pp. 154-155), but he says in his personal correspondence addressed to Mr. H. SATÔ of our Institute "THEEL's *Caudina arenata armata* shows, in general, a similar arrangement of respiratory trees of that of *C. arenata* GOULD, but a small (4 mm. long) bifid, supernumerary trees open into the cloaca on the posterior and ventral side of the base of the right tree, and three others of similar size open into the cloaca posterior and ventral to the left tree, close to one another along an anterior-posterior ventral line."

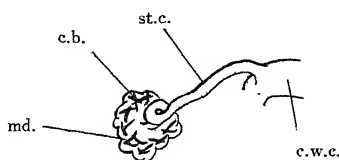
Here I should like to add that a holothurian which was collected by Mr. S. OHFUCHI, at Kesenuma, Miyagi Prefecture, and which may be identified with the species belonging to the genus either *Caudina* or *Molpadia*, also has six supernumerary trees, in this respect resembling *Caudina chilensis*. It may also be here mentioned that the species *Molpadia roretzii* (v. MARENZELLER) which may be found in the waters near the Biological Station of Asamushi, is not provided with such supernumerary trees as is the case with other holothurians closely related to it, but it has two main respiratory trees, each giving off a branch posteriorly at a point slightly distant from the base of these trees.

### 5. Calcareous Ring

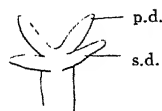
In this stage the calcareous ring is more grown than in the previous stage, and its diameter measures about 0.9–1.6 mm.

### 6. Water-vascular System

The stone canal (text-fig. 27, *st.c.*) is fairly strongly twisted, and the madreporite (*md.*), which is attached to its extremity, is rosette-like in shape.



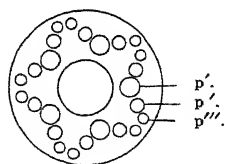
Text-fig. 27. *Caudina chilensis* (J. MÜLLER). The stone canal and the madreporite of the young in the fourth stage.



Text-fig. 28. *Caudina chilensis* (J. MÜLLER). The tip of the tentacle of the young in the fourth stage.

Each of the tentacles is furnished at its tip with four digits of similar appearance (text-fig. 28), and the tentacular ampulla is very much elongated in this stage.

In the early period of this stage there exist fifteen anal papillae placed in five groups, each consisting of three papillae, i.e. a large primary papilla and two secondary ones (text-fig. 22). In the more advanced period of the same stage, two more tertiary papillae (text-fig. 29, *p'''*.) may be added to each group, and thus the number of papillae attains to twenty five in all. In each group the largest primary papilla is situated close to the anus, while the smaller two secondary and two tertiary ones are placed outside the primary, in such a manner as shown in text-figure 29.



Text-fig. 29. *Caudina chilensis* (J. MÜLLER). Diagrammatic illustration of the arrangement of the anal papillae.

### 7. Blood-vascular System

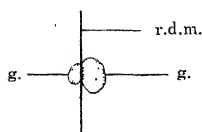
In this stage, the blood capillaries (Pl. III, fig. 12, *cap.*) are conspicuously developed, and are mingled with the fine branches of the left dorsal branch of the left respiratory tree.

The efferent vessel also has put forth many side-branches. Further, the commissures have increased and are from three to five in number.

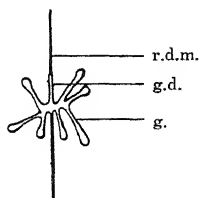
### 8. Genital Organ

The rudimentary gonad which first appeared in the right dorsal mesen-

tery is subsequently constricted into two portions, both being attached to the above (Pl. III. fig. 13, text-fig. 30, *g.*).



Text-fig. 30. *Caudina chilensis* (J. MÜLLER). Diagram to show the gonad of the young about 13 mm. long.



Text-fig. 31. *Caudina chilensis* (J. MÜLLER). Diagram to show the gonad of the young about 30 mm. long.

At the end of this stage each gonad gives off branches which grow larger, and afterwards a short gonoduct (text-fig. 31, *g.d.*) common to both gonads appears between two layers of the right dorsal mesentery.

The coelo-anal canal which was first mentioned by KAWAMOTO as existing in the adult of the present holothurian (1927, p. 244) is not yet formed in such young forms as are dealt with in the present paper.

#### SUMMARY.

1. The young forms of *Caudina chilensis* after their embryological stage are divided into four groups. The first, second, third, and fourth stages are represented by individuals of about 1-2 mm., 2-4 mm., 4-10 mm., and 10-30 mm. in total body length, respectively, and the tentacles are peculiar to every stage as regards their shape and number. The young of the last stage show external and internal features almost similar to those of the adult.
2. The tail of the young is, at first, very short, but is gradually elongated. Sand grains cover the body surface all over in the first, second, and third stages, but in the fourth stage, the area covered by the sand grains is gradually diminished, until, finally it remains only at the proximal end of the tail.
3. As in the case of the adult, the body wall consists of four layers, *i.e.* an outer epithelium, a layer of connective tissue, a muscular layer, and a thin inner epithelium.
4. The calcareous bodies are first noticeable in the madreporite and in the connective tissue layer of the body wall. They cover the anal region in the young of the first stage, but in the later stages we also find them in the regions more anteriorly localized.
5. Each of the longitudinal muscles is a single strand in the first stage, but in the next stage it shows some tendency to divide into a pair, and

it becomes very distinct in the fourth stage.

6 The nervous system of the young consists chiefly of the nerve ring and five radial nerves.

7. The alimentary canal of the young is nearly similarly formed as it is in the adult. The features of the pharynx, stomach, and the intestine are almost similar to those of the adult. But the cloaca is very short in the early stage and it becomes gradually longer in the later stages relatively to the elongation of the tail.

8. The peri-stomachal sinus becomes discernible in the second stage.

9. The respiratory trees, the left and the right, which are formed early in the first stage, first of all, have no side branches, but in the fourth stage they are provided with many of these which look like those of the adult.

The supernumerary respiratory trees which are observable in some species in Molpadiidae and in Cucumariidae first appear in the second stage of the young in the form of two bud-like protuberances arising from the base of the main respiratory tree. They are full grown in the fourth stage, and look like those to be seen in the adult.

10. The calcareous ring is first formed in the very young animal in the first stage, and becomes gradually larger, its diameter increasing in the later stages.

11. The water-vascular system is comparatively well-developed in the young. The circular water canal, Polian vesicle, and the radial water canals are almost similarly formed as those of the adult.

The stone canal is almost straight early in the young stage, but it is more or less twisted in the fourth stage.

The madreporite is at first circular and smooth in outline, is studded with a small number of calcareous bodies, and, moreover, is directly attached to the body wall. But in the later stages it becomes rosette-like in shape, is covered with numerous calcareous bodies, and then it is freely set, not adhering to the body wall.

As regards the tentacles, it may be stated as follows: in the first stage the secondary tentacles are formed in addition to the primary ones, and thus they are ten in number, each provided with two digits at the tip. The tertiary tentacles appear in the second stage, and the tentacles are now fifteen in number, and are all furnished with two digits at their tips. In the third stage two smaller secondary digits are added to each of these tentacles. These secondary digits gradually grow larger till they become like those in the primary, and thus, in the fourth stage the tentacles have

an appearance similar to that of the adult.

The ten tentacular ampullae are first formed at the bases of the primary and secondary tentacles in the second stage, and later in the third stage five more of those are formed at the bases of the tertiary tentacles. They are strongly elongated in the fourth stage.

Five anal papillae appear as small projections situated at each radius in the first stage. They are primary papillae. At the end of the second stage, ten secondary papillae are added to the primary ones and then ten tertiary papillae are added in the fourth stage. Thus the number of anal papillae become twenty five in all, and it is observed that they are set in five groups, each consisting of five papillae.

12. The dorsal blood vessel is represented only by the efferent vessel in the first stage. But, in the second stage it is differentiated into two vessels, the afferent and the efferent. In the third stage, a number of capillaries appear arising from the afferent vessel. In the fourth stage, the dorsal vessel is fully developed and gives the appearance of being almost similar to that seen in the adult.

The ventral blood vessel consists, in the first stage, of two vessels, each of which runs along the ventral side of the small intestine. Sooner or later one commissure develops between these two ventral vessels. The number of commissures increases in the advancing stages of growth. In the second, third, and fourth stages, there are from one to two, from two to four, and from three to five commissures, respectively.

13. The gonad is formed first in the third stage as a small rounded body involved in the right dorsal mesentery. This body is constricted into two portions in the beginning of the fourth stage, and each grows larger giving off a number of branches. The rudimentary gono-duct is formed at the end of this stage.

14. The coelo-anal canal is not observable in the young.

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### EXPLANATION OF THE PLATES.

#### Pl. II.

- Fig. 1. *Caudina chilensis* (J. MÜLLER). The body wall of the young about 2 mm. long.  $\times 500$ .  
Fig. 2. The same. The cross section of the oral region of the young about 1.8 mm. long.  $\times 100$ .  
Fig. 3. The same. The cross section through the pharynx of the same as fig. 2.  $\times 100$ .  
Fig. 4. The same. The cross section through the middle portion of the body of the young about 2 mm. long.  $\times 100$ .  
Fig. 5. The same. The cross section through the stomachal region of the young about 3.3 mm. long.  $\times 60$ .  
Fig. 6. The same. The longitudinal section through the buccal region of the young about 3.5 mm. long.  $\times 280$ .  
Fig. 7. The same. The cross section of the body wall of the young about 6 mm. long.  $\times 300$ .

#### Pl. III.

- Fig. 8. *Caudina chilensis* (J. MÜLLER). The cross section of the dorsal blood vessels of the young about 6 mm. long.  $\times 100$ .  
Fig. 9. The same. The cross section of the gonad of the young about 8 mm. long.  $\times 300$ .  
Fig. 10. The same. The cross section of the body wall of the young about 30 mm. long.  $\times 100$ .  
Fig. 11. The same. The ventral view of the cloaca of the adult about 225 mm. long.  
Fig. 12. The same. The cross section of the rete-mirabile of the young about 20 mm. long.  $\times 60$ .  
Fig. 13. The same. The cross section of the gonad of the young about 13 mm. long.  $\times 300$ .





Fig. 1.

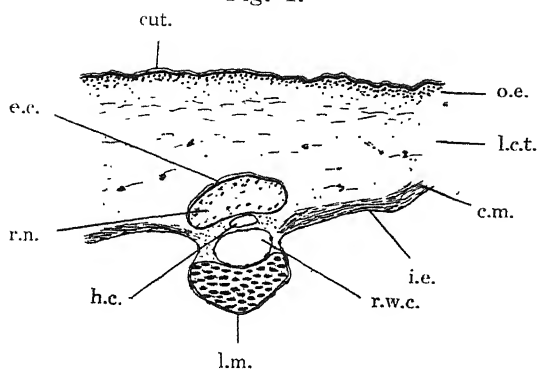


Fig. 7.

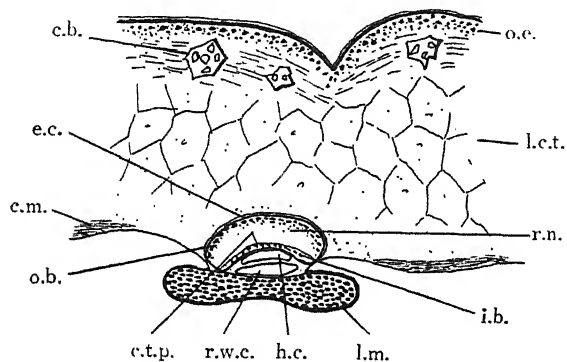


Fig. 2.

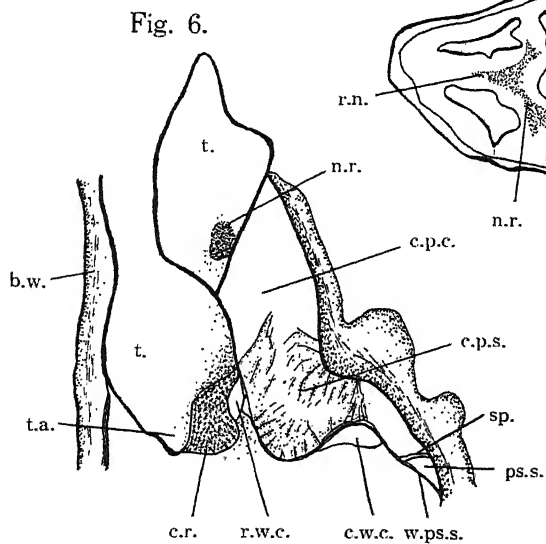


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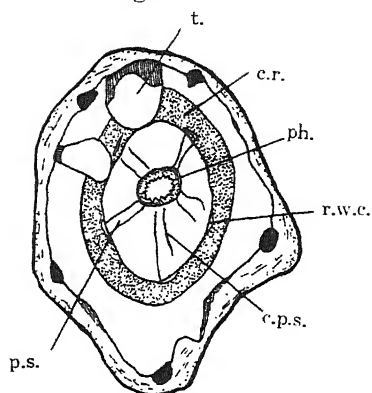


Fig. 5.

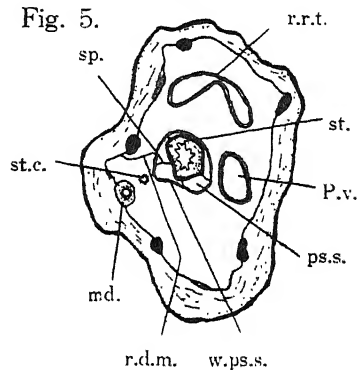


Fig. 4.

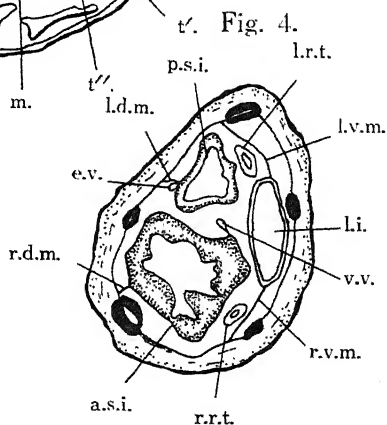




Fig 9

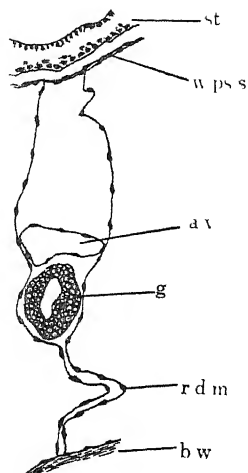


Fig 10.

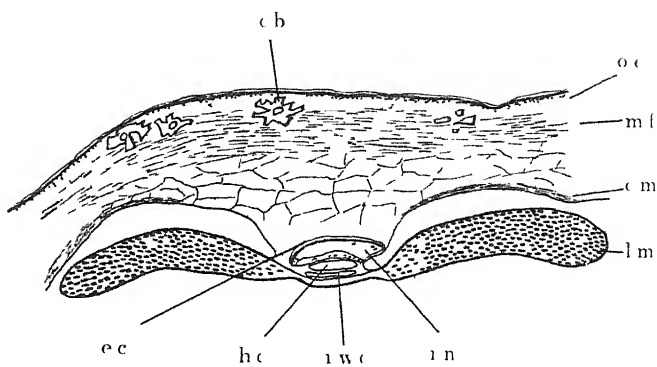


Fig 12

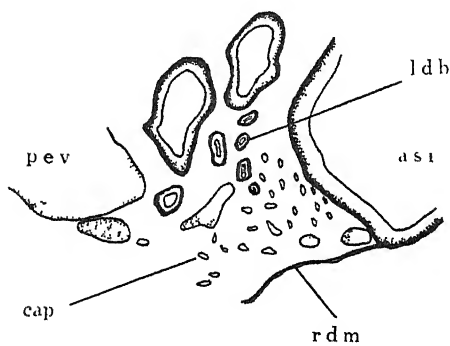


Fig 13

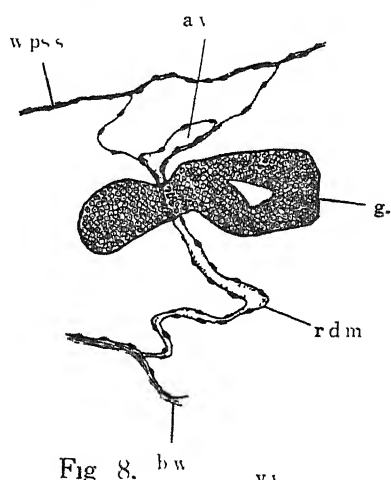


Fig 8.

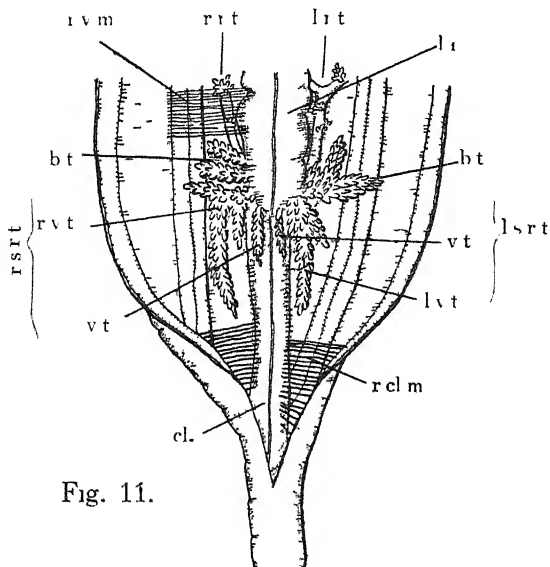
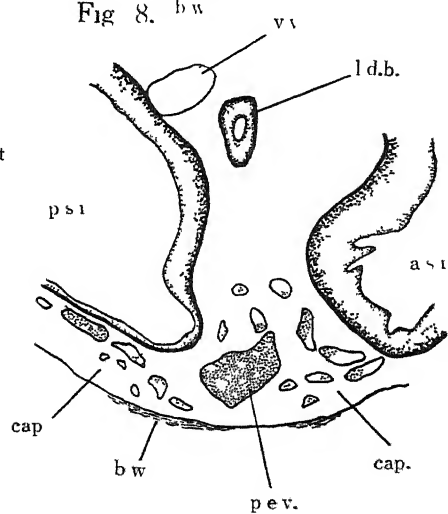


Fig. 11.



# CONTRIBUTIONS TO THE PHYSIOLOGY OF THE ASCARIS.

## 1. GLYCOGEN CONTENT OF THE ASCARIS, *ASCARIS MEGALOCEPHALA* CLOQ.

By

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(With Pl. IV, and 1 text-figure).

(Received November 30, 1932)

### INTRODUCTION.

Intestinal worms are the only multicellular animals known which normally exist in absence of oxygen. Although they have no need to procedure heat, they require energy for other purposes, such as muscular movement, growth, and so on. They are, therefore, very instructive for investigation of anaerobic changes because of the fact that they live in the small intestine, where oxygen is almost absent and accordingly they are supposed to consume the glycogen stored in their bodies for their life phenomena.

The most recent work on the glycogen content of ascaris is that of WEINLAND (1901-1906). He found that *Ascaris lumbricoides* contains a large quantity of glycogen (4.2-7.1% in fresh worms and 20-34% in dried matter of the worms) which is consumed in starvation, giving as product, in absence of oxygen, carbon dioxide and valerianic acid. As far as I know, however, there is no who has studied the anaerobic changes in *Ascaris megalocephala*.

The present work was undertaken to determine the glycogen content and its distribution in the ascaris, *Ascaris megalocephala* CLOQ., as the first step of investigations concerning the anaerobic changes of the animal.

### MATERIALS AND METHODS.

The ascarides used in my experiments were found in the small intestine of horses employed for the anatomical researches for students in the Morioka Imperial College of Agriculture and Forestry. Some of the ascarides were also collected from the Morioka Slaughter-House. In this experiment only fresh, healthy specimens, varying in weight from about 2 to 8 gms. in the females and from 2 to 3 gms. in the males, were used.

The technique employed in the analytical procedure for the estimation

of glycogen in the whole body was PFLÜGER's method. The distribution of glycogen in the tissues of the worm was examined not only by a micro-chemical procedure, but also by a histological procedure. In the latter case, BEST's carmin stain was used for the tissues after fixation in CARNOY's fluid. The ascarides collected were immediately used, since this investigation requires the materials under the possible freshest condition. The worm was weighed directly after quickly wiping the body surface with a clean cheese cloth, and the tissues were separately dissected in a small shallow porcelain dish and weighed indirectly by difference in the weight of the dish with and without the sample. The samples thus obtained were digested in a 60 per cent solution of potassium hydroxide on a steam bath and the glycogen dissolved in it was precipitated by 70 per cent alcohol. The estimation of glucose following the acid inversion of the isolated glycogen from the whole body of the ascaris was made by BERTRAND's method. FOLIN-WU's colorimetric procedure, however, was used for the micro estimation of the converted glucose from glycogen in tissues of the ascaris.

#### RESULTS OF THE EXPERIMENT.

I. *Result obtained for the glycogen estimation on the whole body.* Glycogen content in the whole body of the ascaris, was determined from January to November, 1931. The result is as follows: In Table I are given the results obtained in the fresh females, in Table II the results in the fresh males, and in Table III the results obtained in the dry matter of the worm.

TABLE I.

*Glycogen contents in the whole bodies of fresh female ascarides.*

Date (1931)	Body weight in gms.	Glycogen in mgms.	Percentage
Feb. 8.	1.70	40.780	2.400
"	2.15	65.550	3.002
"	2.55	70.452	2.763
"	4.18	161.444	3.862
"	4.40	149.320	3.394
April 20.	6.73	233.715	4.229
"	6.81	317.625	4.664
"	8.12	375.335	4.622

Date (1931)	Body weight in gms.	Glocogen in mgms.	Percentage
June 12.	4.00	157.590	3.939
"	4.18	179.444	4.261
"	5.15	211.150	4.100
July 25.	4.36	141.254	3.237
"	4.75	153.820	3.236
Nov. 11.	3.65	137.600	3.770
"	3.84	157.330	4.037
"	6.00	232.004	3.867
July 6. (1932)	4.18	179.444	4.261
Average			3.747

TABLE II.

*Glycogen contents in the whole body of fresh male ascarides.*

Date (1931)	Body weight in gms.	Glycogen in mgms.	Percentage
Feb. 8.	1.68	51.404	3.128
"	1.70	49.595	2.912
"	1.85	56.547	3.057
April 20.	1.40	34.703	2.478
"	1.55	39.318	3.417
"	2.40	72.940	3.039
June 12.	0.90	31.520	3.502
"	1.50	33.307	2.220
Nov. 11.	1.50	36.850	2.456
"	1.75	54.482	3.113
Average			2.917

As seen in Table I, II and III, *Ascaris megalcephala* contains a great amount of glycogen. Comparing Table I with Table II, there appears a wide variation in the glycogen content between the female and the male, the former showing a greater amount than the latter. The value in the female ascaris is on the average about 3.75 per cent in the fresh, whole body, corresponding to 23 per cent in the dry matter of the worm, and that in the male ascaris is on the average about 2.9 per cent in the fresh, whole body, corresponding to 15 per cent in the dry matter.

KOBAYASHI and OKASAKI (1929) found that the glycogen content in the oyster varies widely, not only with the seasons but also with the



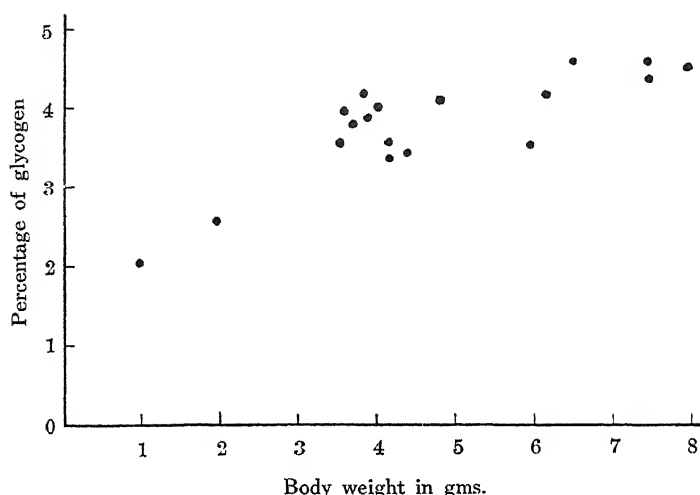


Fig. 1. Showing wide variations in the glycogen content among the individuals of female ascarides.

individuals. WEINLAND (1901) observed that the glycogen content in *Ascaris lumbricoides* shows no variation with the seasons nor with the individuals. He estimated about 5.5 per cent for summer and 5.3 per cent for winter. In my experiment on *Ascaris megalocephala*, no seasonal

TABLE III.

Glycogen content in the dry matter of *Ascaris megalocephala* and water content in the worm.

Date (1932)	Body weight in gms.	Dry matter in gms.	Glycogen content in the dry matter in gms.	Percentage of glycogen in the dry-matter	Water content in gms.	Percentage of water content
April 25	6.725	1.284	282.735	20.020	5.445	80.920
"	6.730	1.276	329.085	25.781	5.454	81.040
"	7.249	1.502	319.875	21.296	5.747	79.280
"	8.220	1.600	375.335	23.458	6.620	80.540
July 6	2.168	0.418	96.086	22.954	1.752	80.813
"	2.169	0.418	89.873	21.552	1.753	81.236
"	2.285	0.473	105.875	22.151	1.812	79.300
"	3.504	0.680	166.860	24.515	2.824	80.594
"	4.114	0.803	194.670	24.367	3.311	80.481

Date (1932)	Body weight in gms.	Dry matter in gms.	Glycogen content in the dry matter in gms.	Percentage of glycogen in the dry matter	Water content in gms.	Percentage of water content
July 6	4.254	0.810	198.305	24.593	3.444	80.959
♀ Average				23.068		
April 25	1.862	0.449	53.303	14.099	1.413	75.886
"	2.104	0.480	63.962	13.321	1.624	77.186
"	2.120	0.487	73.236	15.038	1.633	77.028
"	2.308	0.557	88.992	15.977	1.753	76.127
July 6	1.275	0.255	38.934	17.980	1.020	80.000
♂ Average				15.283		

variation is found in both the female and the male. There are, however, wide ranges of variations in the content of glycogen among the individuals of female ascarides, showing a greater amount of glycogen in the adult specimen than in the young one, as is shown in Table I. and Fig. 1. No remarkable variations in the glycogen content among the male individuals are found as seen in Table II.

It seems highly probable that a great amount of glycogen found in a large female ascaris is associated with the glycogen stored in the reproductive system of the worm. This will be fully discussed under the section "Result obtained for the distribution of glycogen."

TABLE IV.

Host	Parasite	Glycogen content in %	Investigators
Sheep	<i>Tenia expansa</i>	1.5- 4.7 (in fresh body) 15.0-47.0 (in dry matter)	WEINLAND
Human and pig	<i>Ascaris sp.</i>	2.0 (in fresh body)	FOSTER
	<i>Ascaris lumbricoides</i>	4.2- 7.1 (in fresh body) 20.0-47.0 (in dry matter)	WEINLAND
Horse	<i>Ascaris megalcephala</i> (♀)	2.4- 4.7 (in fresh body) 20.0-25.0 (in dry matter)	TORYU
	" (♂)	2.2- 3.4 (in fresh body) 13.0-17.0 (in dry matter)	
"	<i>Styrongylus vulgaris</i>	3.5 (in fresh body)	TORYU
"	<i>Filaria equina</i>	2.4	TORYU
		2.0	

To compare the glycogen content in *Ascaris megalocephala* with that of several other parasites which had been determined by several investigators, Table IV is given.

As is shown in Table IV, glycogen content in *Ascaris megalocephala* is about 4.7 per cent at the highest and never so high as the value in *Ascaris lumbricoides* found by WEINLAND, but is of much the same order as that found for *Ascaris* sp. by FOSTER and that for *Tenia expansa*. It will be noticed in the same Table that the glycogen content in *Ascaris*, *Strongylus* and *Tenia* living in the intestine is greater than that in *Filaria* living in the coelom of the horse.

The variations in glycogen content among several kinds of worms given in Table IV are probably due to the specificities of the worms used on one hand, and to the nature of liquid around them owing to the diet of their host or to the living place of the worms on the other hand.

As regards water content in *Ascaris*, WEINLAND (1901) estimated from 79.5 to 80.1 per cent of water in *Ascaris lumbricoides*. In my experiment in *Ascaris megalocephala*, as is shown in Table III, water content in the female is about 79 to 80 per cent, showing a good agreement with that of *Ascaris lumbricoides* found by WEINLAND just stated. The male contains a somewhat less amount of water than the female, estimating about from 75 to 80 per cent.

II. *Result obtained for the distribution of glycogen.* The data obtained from 8 individuals in chemical procedure are separately given in Table V.

FOSTER (1865) suggested that the glycogen in ascaris is chiefly stored in the muscle. In my experiment on *Ascaris megalocephala*, as will be seen from Table V, the amount of glycogen contained in the muscle of the female is on the average about 5.8 per cent of the muscle weight or about 70 per cent of the total glycogen in the whole body, and that of the male is estimated on the average about 5.0 per cent of the muscle weight, corresponding to 95 per cent of the total glycogen in the whole body. In the female ascaris, glycogen is also contained in the reproductive system, especially in the ovary; the amount in which varies widely with the individuals, varying from about 4.7 to 9.5 per cent of the tissue weight or from about 15.0 to 30.0 per cent of the total glycogen in the worm; the large one shows a greater value than the small one. Reproductive system of the male, however, contains only a very small amount of glycogen.

From the result obtained in the ovary just stated, I consider that the female ascaris contains a greater amount of glycogen than the male ascaris as already stated is mostly due to the fact that the reproductive organ of

TABLE V.

Body weight in gms.	Tissue	Tissue weight in gms.	Glycogen content in mgms.	Percentage	Percentage against the total glycogen
3.8 (♀)	Muscle	1.195	56.304	4.783	64.436
	Intestine	0.350	1.125	0.322	1.288
	Ovary	0.460	21.113	4.546	24.161
	Uterus	0.580	8.840	1.345	10.116
3.92(♀)	Muscle	1.20	69.060	5.750	53.772
	Intestine	0.38	2.450	0.645	1.908
	Ovary	0.41	26.800	6.537	20.861
	Uterus	0.61	9.120	1.495	7.100
4.0 (♀)	Muscle	1.77	139.996	7.910	71.129
	Intestine	0.46	5.840	1.269	2.967
	Ovary	0.40	26.664	7.416	15.072
	Uterus	0.90	21.321	2.369	10.833
4.9 (♀)	Muscle	1.92	104.288	5.431	75.522
	Intestine	0.52	3.115	0.599	2.256
	Ovary	0.39	17.673	4.516	13.523
	Uterus	0.81	13.000	1.605	9.636
6.8 (♀)	Muscle	2.38	112.000	5.126	56.593
	Intestine	0.90	3.806	0.435	1.923
	Ovary	0.74	70.928	9.463	35.840
	Uterus	1.02	11.170	1.095	5.644
1.37(♂)	Muscle	0.74	32.900	4.446	95.807
	Intestine	0.15	0.640	0.427	1.864
	Reproductive system	0.17	0.800	0.781	2.327
1.39(♂)	Muscle	0.74	36.640	4.951	97.577
	Intestine	0.13	0.810	0.623	2.157
	Reproductive system	0.16	0.100	0.063	0.266
1.49(♂)	Muscle	1.05	55.620	5.297	96.168
	Intestine	0.20	1.391	0.695	2.340
	Reproductive system	0.12	0.825	0.695	1.427

Muscle . . . including cuticula, subcuticula and muscle cells.

Intestine . . including oesophagus, mid and hind guts.

Reproductive system . . including testis, vas deferens, seminal vesicle and ejaculatory duct.

the female contains a great amount of glycogen, while that of the male contains only a small amount, and furthermore that the value of glycogen content in the large female is higher than that in the male is also closely associated with more or less amount of glycogen stored in the reproductive system, especially in the ovary of the worm used.

III. *Result obtained in a histological procedure for the distribution of glycogen.* The result obtained in analytical result was tested by a histological procedure. As a fixing agency CARNOY's fluid was used. Celloidin for imbedding were employed to prevent the glycogen from dissolving into water. Sections were taken through the regions containing sexual organs, and were stained with BEST's carmin fluid. Microscopic examination was made on the sections thus treated.

Stained with BEST's carmin solution glycogen appeared microscopically in the form of small red granules. Contrasting amount of the glycogen granules in tissues given in Table VI shows a good agreement with the analytical result as already stated.

TABLE VI.  
*Contrasting amount of glycogen content in tissues.*

Sex	Body wall			Genital organ			
	Cuticula	Sub-cuticula	Muscle	Oogonia or spermatocyte	Oocyte or spermatocyte	Ovum or sperm	Intestine
♀	—	++	##	##	##	+	+
♂	—	++	##	+	—	—	+

##, ++, +, —... Descending degrees of the amount of glycogen.

Microscopically the non-contractile substance of the muscle cells are filled with glycogen granules, uniformly distributing through the cytoplasm, while no glycogen granules are found in the contractile substance of the cells (Fig. 1).

Remarkable variations are observed in the glycogen content among the cells in the reproductive organs of the female ascaris. Developing young cells, such as, oogonia and oocyte, are richly supplied with glycogen granules so that the whole cut surface of the section of the ovary assumes an intense red color (Fig. 1.), adult cells in the uterus, however, store a small amount of glycogen (Fig. 3.). Glycogen granules are also seen in the cytoplasm of spermatogonia, though the amount is considerably less than

in the cytoplasm of oogonia. No glycogen is seen in the cytoplasm of spermatocytes and spermatids in the vas deferens and the seminal vesicle, showing a negative reaction with BEST's carmine stain. Flagelated epithelial cells on the wall of vas deferens and seminal vesicle are supplied with glycogen in some measure as in the epithelial cells of uterus.

A small amount of glycogen is also observed in the epithelia of the intestine, where glycogen granules are located in the central portion of the cylindrical cells of the intestine.

It was necessary to test whether the red granules in tissues stained with BEST's carmine are glycogen or not, because BEST's carmine method also stains the mucin in certain cells and some granules which are not glycogen. For this purpose, some of the sections were immersed for 12 hours in a 1 per cent solution of panctase (SATO), and then stained with carmine. In sections thus treated, no red granules were found (Fig. 4).

#### SUMMARY.

The results obtained in this investigation may be summarized as follows :

1. The female contains a greater amount of glycogen than the male; namely, the female shows on the average about 3.8 per cent of it in the fresh whole body, corresponding to 23 per cent in the dry matter of the animal, while the male shows 2.9 per cent in the fresh whole body, corresponding to 15 per cent of the dry matter.

2. There are wide ranges of variations in the content of glycogen among the female individuals; the adult ascaris contains a greater amount of glycogen than the young one. In the male, however, no remarkable variations are found.

3. The glycogen content in the ascaris shows no seasonal variation.

4. The greatest amount of glycogen in the ascaris is stored in the non-contractile substance of the muscle cells, estimating about 70 per cent of the total glycogen in the female and 95 per cent in the male, while the contractile substance of the muscle cells contains no glycogen.

5. Reproductive system of the female ascaris also stores a great amount of glycogen, estimating about 20 per cent of the total glycogen in the worm; the greatest amount is stored in the developing young eggs. Reproductive system of the male, however, contains only a small amount of glycogen.

Before leaving the subject, I wish to express my hearty thanks to Prof. S. HATAI for his valuable suggestions and criticism throughout the

the entire course of this work. My thanks are also due to Mr. H. IWATA, Prof. of The Morioka Imperial College who helped me with much kindness during this work and to Mr. H. TSUJI who helped me to take the microscopic photograph.

This work is executed with the aid of "The SAITO Gratitude Foundation".

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#### EXPLANATION OF PLATE

- Fig. 1. Ovary.  $\times 100$ . (Oogonia and oocytes are richly supplied with glycogen).
- Fig. 2. Body wall.  $\times 80$ . (Non-contractile substance of the muscle cell containing a great amount of glycogen is surrounded by contractile substance containing no glycogen.)
- Fig. 3. Uterus.  $\times 80$ . (Adult eggs contain a small amount of glycogen.)
- Fig. 4. Body wall treated with 1% solution of pancrease.  $\times 80$ . (Glycogen is digested.)

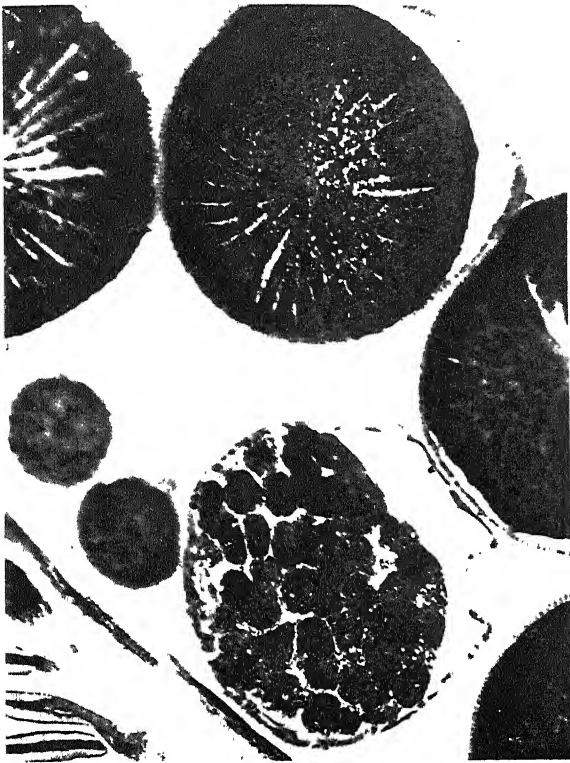


Fig. 1.



Fig. 2.

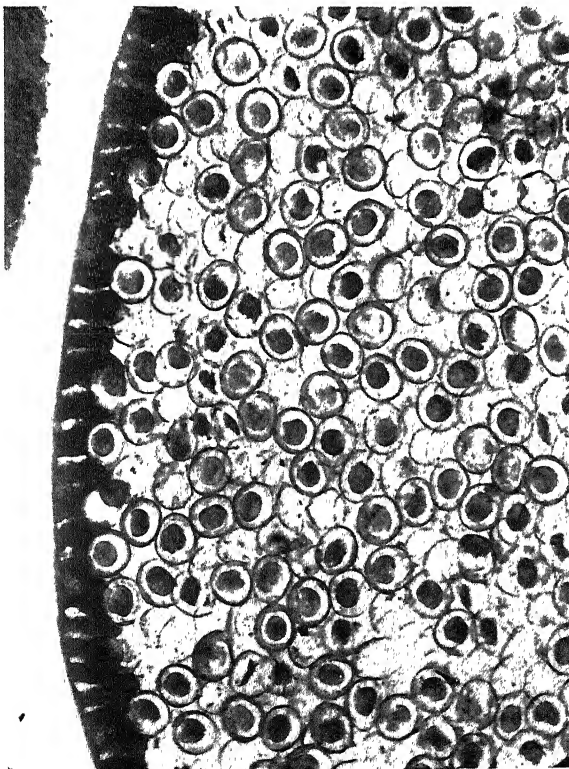


Fig. 3.

Author photo.

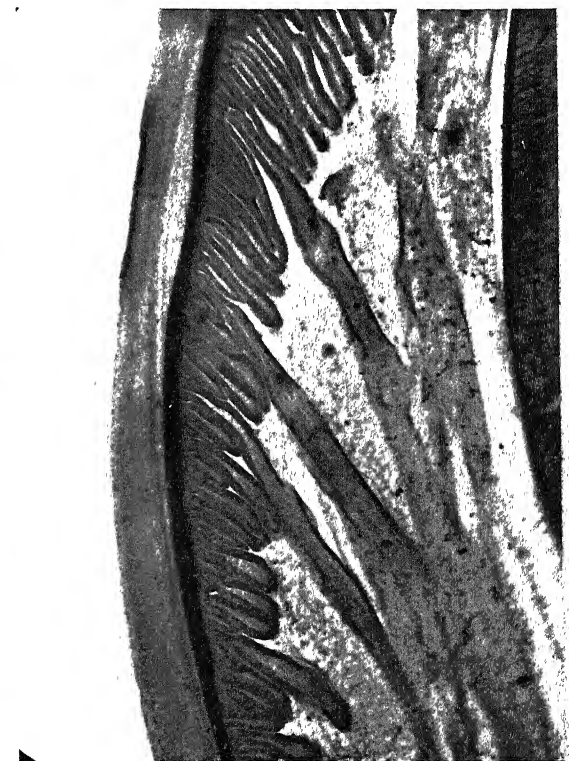


Fig. 4.

Y. TORYU: Glycogen content of the ascaris.





# RECONSTITUTION IN *HALICLYSTUS AURICULA* CLARK.<sup>1)</sup>

By

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Laboratory, University of Chicago.*

(With 29 text-figures)

(Received December 5, 1932)

During the months of July and August, 1931, which were spent at the Marine Biological Laboratory of Tôhoku Imperial University near Asamushi, Japan, the sessile medusoid scyphozoan, *Haliclystus auricula* CLARK. was found in considerable numbers on brown algae near the laboratory. In the course of experiments with the species it was discovered that it possesses rather remarkable capacity for reconstitution. Further experiment showed that the development of a new individual from an isolated piece in this species is of interest not merely as a case of reconstitution in a medusoid scyphozoan, but also from the comparative standpoint in relation to the general conception of reconstitucional development. The experiments also bring to light certain facts of importance concerning physiological dominance.

I desire to acknowledge my deep indebtedness to Dr. HATAI, Director of the laboratory and to Dr. KOKUBO and other members of the staff for placing at my disposal the facilities of the laboratory and for many personal kindnesses.

## MATERIAL.

The animals were fairly abundant on brown algae along shore in the vicinity of the laboratory. The largest individuals were 20–25 mm. in length when extended and about 15 mm. in marginal diameter of umbrella, the smallest used as experimental material, 10–12 mm. in length and 7–8 mm. in marginal diameter. Fig. 1 is a diagrammatic outline of the animal showing the eight adradial marginal lobes bearing the tentacle groups, the eight marginal sensory-adhesive organ-complexes, commonly called marginal anchors (*an*). Four of these are perradial, four interradial. The gonads (*go*) of one side of the body and two of

<sup>1)</sup>Contributions from the Marine Biological Station, Asamushi, Aomori-ken, No. 99.

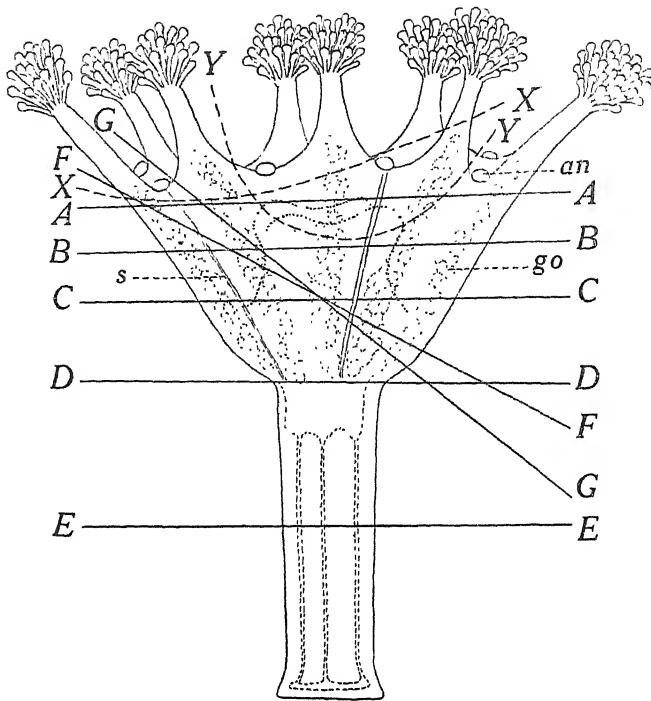


Fig. 1. — Diagrammatic outline of *Haliclystus*. AA-EE, various levels of transverse section; FF, GG, approximate plane of section in two cases of oblique section; XX, YY, Line of section in two cases of removal of a few marginal organs; an, marginal anchor; go, gonad; S, septum.

the interradial septa (*s*) are also indicated in the figure. The manubrium is outlined in dotted line. In description of results of experiment the various marginal organs are distinguished as: *marginal lobes* bearing the *tentacle-groups*; *marginal anchors*, the sensory-adhesive organ-complexes between the marginal lobes. These organs correspond in position to the perradial and interradial tentacles of the scyphistoma and the tentaculocysts of other scyphozoa and are undoubtedly homologous to them. When the animals are in good condition the tentacle-groups show almost continuous movement and the marginal lobes may also contract and extend or bend. The whole umbrella and stalk are also highly contractile; the subumbrellar cavity may be almost completely closed by contraction of the margin; the umbrella may be everted; the whole body may undergo sudden jerking contractions, chiefly longitudinal but usually with circular contraction of the margin; these sudden contractions occur in response to mechanical

or other stimuli and often without perceptible external stimulation.

In general the animals lived in the laboratory up to three weeks and in some cases four weeks without feeding. They were kept in standing water either singly or several together in bowls holding about 250 cc., covered with glass plates to decrease evaporation or in lots of ten in larger containers. Under these conditions it was observed that the death rate was usually lower when the water was not changed during a week or more and change of water was often followed by death. It was not determined what factors were involved in this apparently injurious effect of fresh sea water. In all cases animals were used for experiment within at most a few hours after collecting. Feeding was not attempted and in the absence of food a rapid decrease in size occurs, particularly in the animals undergoing reconstitution. Even in the absence of food, however, individuals of essentially normal proportions but of much smaller size than the original animal often resulted from reconstitution, but in some cases, particularly at the more proximal body-levels, reconstitution was slow and incomplete and reduction of the animal to small size, sometimes only 2-3 mm. in length, may occur without attainment of normal proportions.

The operations consisted in: (1) transverse section at different levels of umbrella and stalk; (2) oblique section through the umbrella with removal of all tentacle-groups and marginal anchors; (3) oblique section through the umbrella with removal of 5-7 tentacle-groups and 5-8 marginal anchors; (4) removal of 2-4 tentacle-groups and 1-3 marginal anchors. Losses of material from high temperature occurred at times during August and by the end of that month the animals had almost completely disappeared from the localities in which they had previously been found. This disappearance may have been due to high temperature of the surface water.

Dilute sea water, 75 and 40 per cent, was used with two lots, ten each, of stalks separated from the umbrella and observations on differential susceptibility to KCN, tap water, distilled water and methylene blue and on the indophenol reaction were made.

All figures of reconstitution are drawn from individuals but are more or less diagrammatic. No attempt has been made to show the exact number of tentacles in the original tentacle-groups or in reconstituted groups consisting of a considerable number of tentacles, but in all figures of earlier stages and of cases in which the reconstituted groups are represented by one, or a small number of tentacles the exact number is

shown in the figures. Also the number and relative sizes of the marginal anchors are shown in each case. Since the paper is primarily concerned with the general form changes and the development and relations of the marginal organs, rather than with the internal organization, gonads and septa are not indicated in most of the figures.

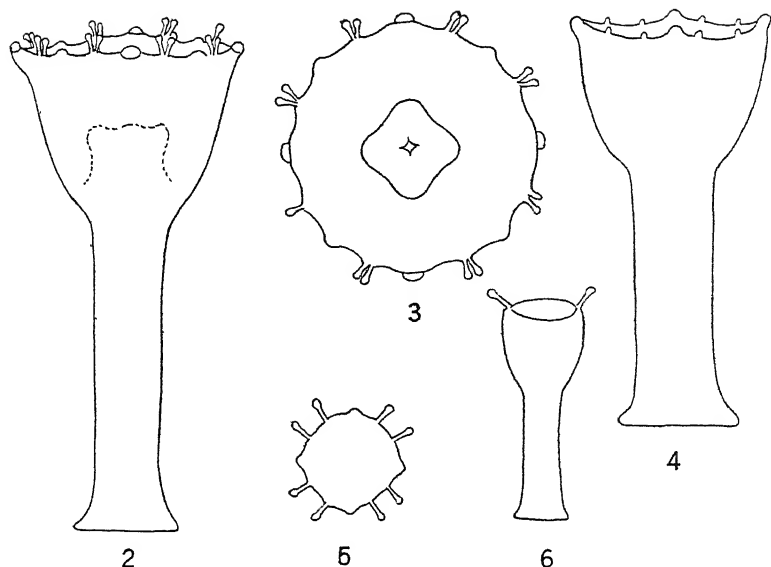
#### EXPERIMENTAL.

*Transverse section at different levels of the umbrella.* A total of 56 animals were divided by transverse section at various levels of the umbrella as indicated approximately by the lines *AA*, *BB*, *CC* in Fig. 1. In no case did the distal pieces show any indication of reconstitution of parts removed or of any reorganization, so far as could be determined and all of them died within 2-3 days after section. Of the 56 proximal pieces 32 died within 3-5 days after section. Thirty of these deaths occurred in early experiments in which all animals in certain containers died. Among the 26 pieces of later series only two deaths occurred before development had reached advanced stages.

When only the distal portion of the umbrella is removed the new tentacle-groups and marginal anchors are visible on the distal margin of the proximal piece in two to three days after section and in eight to ten days the piece approaches the normal animal in form but is of smaller size, the marginal lobes are not fully developed and the number of tentacles in each group is less than in the original animal.

The results of transverse section near the middle levels of the umbrella are very similar to those at distal levels, the chief difference being that development of the distal parts is in general slightly less rapid than at the more distal levels of section. Since reconstitution at the distal and middle levels of the umbrella is essentially similar in character to cases at more proximal levels which are described and figured below, figures of the results of section at the distal and middle levels are regarded as unnecessary.

After transverse section at levels of the umbrella proximal to the middle, *CC*, Fig. 1, reconstitution is in general somewhat less rapid than at the middle level. The case showing the most rapid reconstitution is shown in Figs. 2 and 3 at the stage attained three days after section. Two tentacles are present in each group except one and marginal anchors are developing, but the perradial anchors are distinctly in advance of the interradial (Fig. 3). A large part of the manubrium is present in this



Figs. 2-6. — Reconstitution after section in proximal umbrellar region. Fig. 2, most advanced case 3 days after section. Fig. 3 oral view of individual shown in Fig. 2. Fig. 4, usual stage of development 6 days after section. Fig. 5, oral view of an individual 16 days after section; only 4 perradial anchors present; each tentacle group represented by a single tentacle. Fig. 6, an individual which developed only two tentacles and no anchors.

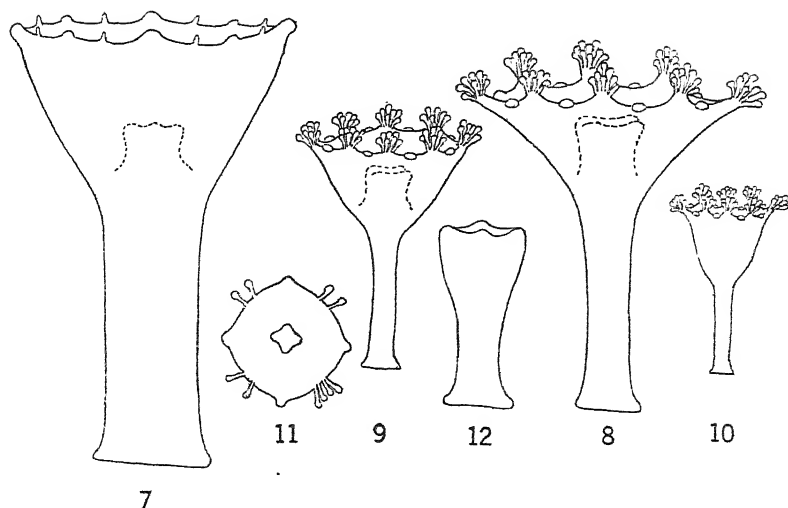
individual because the manubrium contracted strongly at the time of section so that most of its length was proximal to the level of section. Fig. 4 shows the stage usually attained 3-4 days after section. Here only one tentacle in a very early stage of development represents each group and only perradial marginal anchors are present. Pieces like Figs. 2 and 4 continue to develop, the umbrella increases and the stalk decreases in relative size while the whole decreases in absolute size, the tentacles of each group increase in number, the marginal lobes gradually develop and interradial anchors attain the same size as the perradial. Fifteen days after section such animals are much reduced in size, often only 4-6 mm. in length but approach intact animals in form and proportions, have several tentacles in each group, short marginal lobes and the full number of anchors. Some pieces (20 per cent) showed less complete reconstitution. For example, Fig. 5 shows the outline in oral aspect of a proximal piece sixteen days after section in the proximal umbrellar region. A single tentacle represents each group and only perradial anchors are present. No further

development occurred in this case. The case shown in Fig. 6, also sixteen days after section, developed only two tentacles and no marginal anchors. In a third case one tentacle-group, in a fourth, three groups did not develop at all. These cases of incomplete reconstitution do not throw any light on the conditions determining the failure of certain parts to develop.

*Transverse section at the junction between umbrella and stalk.* Thirty individuals were sectioned at the junction between umbrella and stalk (DD, Fig. 1). Of these ten were kept in normal sea water, ten each in 75 per cent and 50 per cent sea water. Of the ten proximal pieces, stalks only, in normal sea water five developed the full number, eight, of tentacle-groups and anchors and five were incomplete, 1-4 tentacle-groups and anchors being absent. Fig. 7 shows the most advanced case six days after section. Each tentacle-group is represented by only one tentacle in an early stage and the perradial anchors are more advanced than the interr radial in development. In only one other piece of the ten were tentacles visible six days after section. Reference to the preceding section and comparison of Figs. 7 and 3 shows that reconstitution at the level of the junction between umbrella and stalk is slower than at the proximal umbrellar level.

Figs. 8, 9 and 10 show individuals reconstituted from stalks sixteen days after section. The differences in size result from differences in original size of the stalk and differences in rate of reduction. Evidently the stalk alone is capable of reorganizing into a complete individual. The umbrella arises, not by the outgrowth of new tissue from the cut surface, but by the reorganization of the distal portion of the stalk.

In the ten stalks in 75 per cent sea water reconstitution was somewhat retarded, at least in the later stages. Six days after section there was no marked difference between the stalks in normal and those in 75 per cent sea water, but sixteen days after section both retardation and development of a smaller number of marginal organs in 75 per cent than in normal sea water were evident. At that time only one animal in dilute sea water showed the full number of tentacles and anchors, as in Fig. 10. Two showed four perradial, no interr radial anchors and four tentacle-groups, each probably double, that is, consisting of two groups close together without anchors between them. Four others were incomplete, one with only two tentacles on opposite sides and no anchors, one with four tentacle-groups and anchors on one side of the margin and none on the other, one with five tentacle groups and five anchors and one with



Figs. 7-12. — Reconstitution of stalk. Fig. 7, most advanced case 6 days after section; perradial anchors more advanced in development than interradian. Figs. 8, 9, 10, individuals reconstituted from stalks at stage 16 days after section. Fig. 11, oral view of stalk reconstitution in 50 per cent sea water for 6 days following section, then 10 days in normal sea water; development of interradian anchors completely inhibited and tentacle-groups approximated to interradii. Fig. 12, maximum development after 16 days in 50 per cent sea water.

no trace of tentacles or anchors although transformation of the distal part of the stalk region into an umbrella had taken place. Three of the ten had died.

In the ten stalks in 50 per cent sea water no reconstitution beyond a slight enlargement of the distal part of the stalk, that is, the beginning of development of an umbrella, took place during six days. At that time five individuals were returned to normal sea water and five remained in 50 per cent. Fifteen days after section only two of those returned to normal sea water were alive. Both showed four tentacle-groups and four perradial anchors (Fig. 11). The internal structure of the animals shows, however, that each of the pairs or double pairs of tentacles represents two tentacle-groups, the interradian parts of the margin and the interradian anchors having failed to develop as in some of the animals in 75 per cent sea water. Figs. 2-5 above show cases of reconstitution in normal sea water in which the interradian anchors are either smaller than the perradial (Figs. 2, 3) or entirely absent (Figs. 4, 5). Figs. 5 and 11 are very similar, but in Fig. 5 the interradian region is less completely inhibited



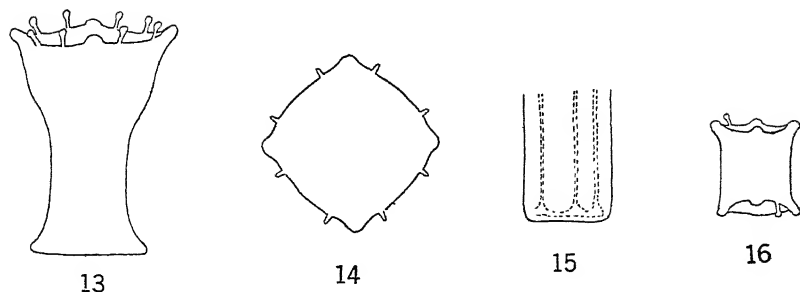
than in Fig. 11. In both of these cases development did not proceed further than the stage of the figures.

Of the five stalks which remained in 50 per cent sea water four were alive after fifteen days. Fig. 12 shows the condition of the most advanced of these. The four perradii are indicated by elevations of the margin which probably represent very early stages of the anchors, but no tentacles are present. The oral surface shows merely a slight depression without manubrium. The three others show merely a slight enlargement of the distal stalk region. According to these few data dilution of sea water retards or almost completely inhibits reconstitution. The point of chief interest, however, is that interradiation development is more inhibited than perradiation. The retardation or inhibition of interradiation development which was observed in normal sea water in some cases including the proximal umbrellar region and in some of stalks only appears in more extreme form in these stalk reconstitutions in 50 per cent sea water.

In none of the thirty cases of section at the junction of stalk and umbrella did the distal pieces, the umbrellas, show any indication of development of a stalk and all of them died within a week.

*Transverse section at middle of stalk.* Twenty animals were sectioned approximately at the middle of the stalk (EE, Fig. 1). All proximal pieces (proximal halves of stalks) were alive after six days, but only three showed tentacles and anchors. Figs. 13 and 14 show the most advanced cases six days after section. Each tentacle-group is represented by a single tentacle, only perradiation anchors are present and the manubrium has not yet developed. The case of Fig. 14 shows more advanced tentacle development than Fig. 7 which consists of the whole stalk, but in Fig. 7 interradiation anchors and manubrium are present, in Fig. 14 absent. Ten days after section 13 of the proximal stalk-halves were alive. Seven of these were small complete individuals 4-8 mm. in length with eight tentacle-groups of one to several tentacles each and eight marginal anchors. These were essentially similar to Fig. 10. In the other six 1, 4, 4, 3, 6, 8 tentacle groups and 1, 4, 4, 0, 4, 6 anchors were absent. In the last case in which all tentacle-groups and six anchors were absent the distal end was slightly enlarged and slightly concave and the two marginal anchors were diametrically opposite. After sixteen days ten of the proximal individuals were alive, five complete individuals. After twenty-five days three were still alive and 3-7 mm. in length.

The distal pieces including umbrella and half the length of the stalk did not die as early as when the level of section was farther distal. After



Figs. 13-16. — Reconstitution from middle of stalk. Fig. 13, most advanced case 6 days after section; only perradial anchors present. Fig. 14, usual stage 6 days after section; only perradial anchors present. Fig. 15, slight development of foot-like structure at aboral end of distal piece consisting of umbrella and distal half of stalk; 10 days after section. Fig. 16, bipolar form from distal half of stalk; 7 days after section.

ten days eleven, after sixteen days ten were still alive but none had developed a foot or become attached. The only indications of proximal reconstitution were a flattening of the proximal end of the stalk and slight changes in the internal structure of that region (Fig. 15). These changes undoubtedly represent the beginnings of development of a foot, but a functional foot did not develop in any case. The extreme slowness and slight degree of aboral as compared with oral reconstitution is of considerable interest but discussion is postponed to a later section.

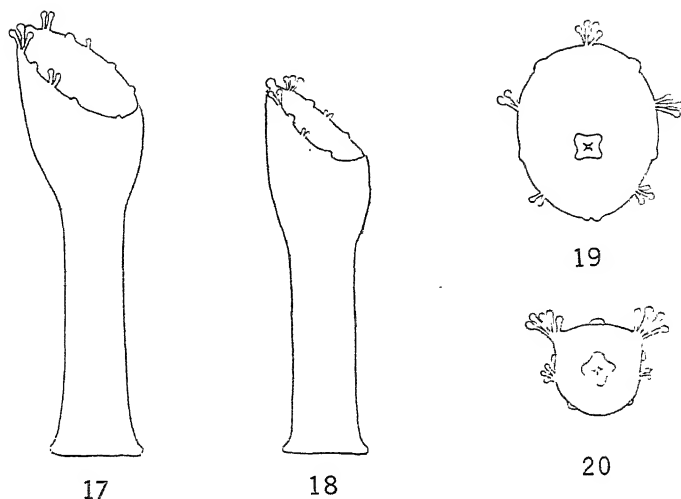
*Transverse section of the stalk into distal and proximal halves.* In seven cases the stalk was separated from the umbrella and then cut into distal and proximal halves. Seven days after section two of the distal halves were alive and one of them was bipolar (Fig. 16). One end showed four perradial anchors developing and a single tentacle, the other end three perradial anchors and also a single tentacle obliquely opposite to the tentacle on the other end. No further development occurred and three days later the piece was dead. The other distal piece became an almost spherical mass without any indication of tentacles or anchors.

Of the proximal stalk-halves four were alive after seven days and showed the following characteristics: (1) 2-3 mm. in length, approaching typical proportions, 8 tentacle-groups, 7 anchors; (2) 3 mm. distal surface slightly oblique, 6 tentacle-groups, one tentacle each, 3 perradial, 2 interradial anchors, all on more distal parts of oblique surface, no development on most proximal part; (3) 2 mm., distal surface oblique, 4 tentacle-groups represented by minute buds on distal half of surface of

section, no development on proximal half; (4) 2 mm., 4 perradial elevations on distal end (as in Fig. 12). In all cases the distal portion of the half-stalk underwent enlargement into the form of an umbrella.

This series was run during the period of high temperature in August and the higher death rate and less complete reconstitution than in the proximal stalk-halves described in the preceding section was undoubtedly due to the temperature. In the hope of obtaining further data, particularly concerning bipolarity other series of distal and proximal stalk-halves and also of thirds were prepared but all pieces died without reconstitution, also apparently from the high temperature.

*Oblique section through the umbrella with removal of all marginal organs.* It soon became evident in the course of the experiments that in general the farther proximal the level of section the slower the reconstitution of the proximal piece and the greater the frequency of incomplete reconstitution. Since the difference in rate appeared to be an expression of an apicobasal gradient oblique sections through the umbrella at various angles removing all tentacle-groups and anchors were made (for example, *FF*, Fig. 1) in the belief that the differences in rate would appear at the different levels of the oblique surface of section in the single piece. In all individuals sectioned in this way the difference in rate appeared. Reconstitution occurred most rapidly on the highest (most distal) portion of the oblique surface. But another still more important result appeared in these cases. Reconstitution not only decreased in rate from the more distal to the more proximal levels, but the stage of development attained was most advanced at the most distal levels and progressively less advanced at more proximal levels and, except in one case in which the plane of section was only slightly oblique, tentacle-groups and anchors did not develop at all on the most proximal levels of the oblique surface of section. Figs. 17 and 18 show cases of this sort six days after section. In Fig. 17 seven anchors and five tentacle-groups are present. The tentacles are most numerous and most advanced in development in the two groups on the upper side of the oblique distal surface and on the lower side three groups are absent. In the case of Fig. 18 five anchors and four tentacle-groups are present and show a similar gradation in size and developmental stage. Fig. 19 shows the distal aspect of another case six days after section. Here six anchors and five tentacle-groups have developed. The three most distal groups are most advanced in development, three groups on the lower (more proximal) are absent and two marginal anchors at the most proximal level are side by side.



Figs 17-20. — Reconstitution after oblique section through umbrella with removal of all parts of original margin. Figs. 17, 18, 6 days after section showing inhibition of marginal organs at most proximal levels of section. Fig. 19, oral view of another case 6 days after section. Fig. 20, individual shown in Fig. 18 as it appeared 16 days after section.

The most interesting result of these experiments, however, is that marginal organs which were absent 6-8 days after section were permanently absent. In no case was development completed at the more proximal levels. Fig. 20 shows the distal aspect of the same individual as Fig. 18 at a stage ten days later (16 days after section). Comparison of the two figures shows that no additional tentacle groups or anchors have appeared during the ten days. The animal retains the bilateral symmetry of the earlier stage. Four days later the condition was the same and at that time disintegration was beginning.

In most cases some decrease in number of marginal organs occurred after 10-12 days and in all such cases it was the smaller less developed organs of the more proximal levels which were resorbed or atrophied, while the largest, most highly developed organs of the most distal levels persisted.

Table I gives the numerical data for tentacle-groups and anchors of the nine individuals which showed reconstitution. The individuals of this series were not kept separately and at 20 days after section the disappearance of some of the tentacle-groups and anchors made recognition of some of the individuals uncertain. It is possible, therefore, that in some cases an error has been made in identifying a particular individual.

TABLE I.

*Reconstitution after removal of all tentacle groups and anchors by oblique plane of section through umbrella. Ten individuals: one showed no reconstitution of marginal organs and is not included in the table.*

Individual number	7 days		20 days	
	Number of tentacle groups developed	Number of anchors developed	Number of tentacle-groups present	Number of anchors present
1	5	5	5	5
2	7	6	6	5
3	8	8	6	6
4	7	6	7	6
5	7	6	5	5
6	7	6	7	6
7	5	6	5	6
8	4	5	2	1
9	7	6	4	5
Totals	57	54	47	45
Percentages of total numbers of tentacle-groups and anchors in intact animals	79	75	65.3	62.5

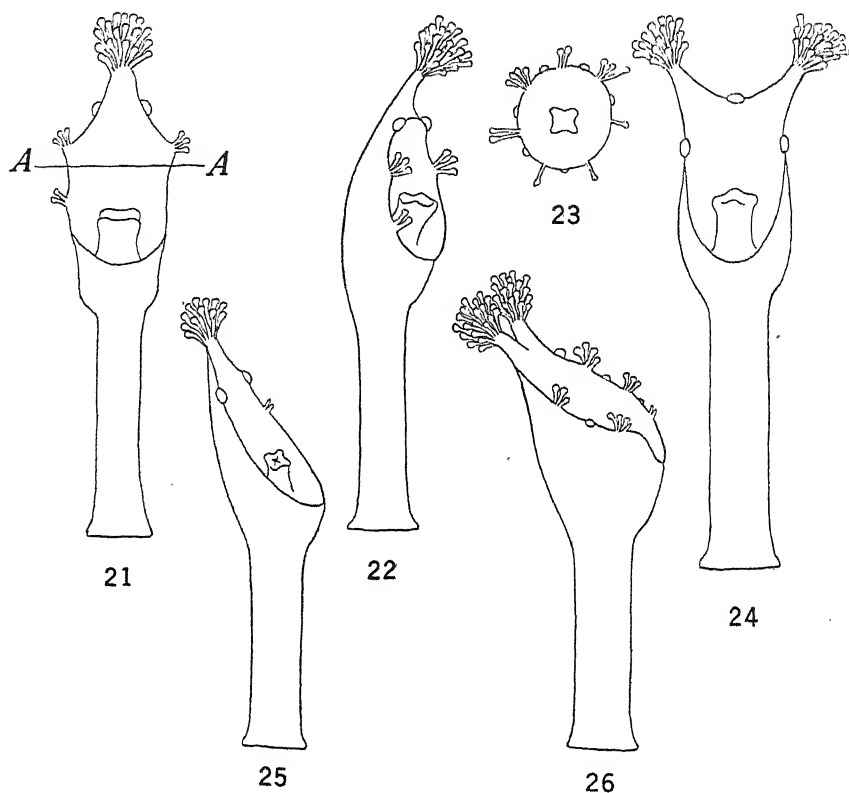
at 20 days with the individual at 7 days. It was determined, however, from observations made between 7 and 20 days that no increase in the number of marginal organs developed occurred after 7 days in any case, consequently such errors do not alter the totals and the percentages given in the table. The table shows that the full number of marginal organs developed in only a single case, No. 3. This was a case in which the plane of section was only slightly oblique. In all others less than the full number of marginal organs developed. At 7 days the totals were 57 tentacle-groups and 54 anchors, 79 per cent and 75 per cent respectively of full normal number, 72. Twenty days after section the totals have decreased to 47 and 45, 65.3 per cent and 62.5 per cent of the normal number. As already noted above, the marginal organs which disappeared were those of the lower more proximal levels. There can be no doubt that the marginal organs of lower, more proximal levels of the oblique surface of section are inhibited in development and the organs which are

most inhibited are those which disappear in later stages. In preceding sections of this paper it has been shown that after transverse section at various levels of the umbrella and even at the junction between umbrella and stalk all the marginal organs may appear within 3-6 days after section and at 6-8 days are always present if they develop at all. In these cases of oblique section the same body-levels which give rise to marginal organs in 3-8 days after transverse section show no development of these organs even after 16-20 days. Discussion of these cases is postponed until further data on the results of oblique section have been presented.

*Oblique section through the umbrella leaving one or more of the tentacle-groups and anchors intact.* In this series of experiments distal parts were removed from 21 individuals by oblique section at various angles in such manner that all but one or two tentacle-groups and one, two, or three anchors were removed. The line GG in Fig. 1 shows approximately the plane of such an oblique section by which all but one tentacle-group and two marginal organs were removed. In all cases of this operation several tentacle-groups and marginal anchors on the lower, that is, the more proximal levels of the oblique margin did not develop at all. The first case of this sort is shown in Figs. 21 and 22, twelve days after removal of all except one tentacle group and two anchors. Reconstitution of the two uppermost tentacle-groups removed and on one side of the animal, of the next lower group has occurred, but these groups have developed much more slowly and consist of fewer tentacles than in cases of transverse section at any level of the umbrella or even at the junction of umbrella and stalk, and the more proximal group on one side developed later than the two upper groups. In the case of the two upper reconstituted groups little more than the marginal lobes was removed, but after twelve days the tentacles of the groups are less developed than in cases of transverse section at proximal umbrellar levels after 3-5 days (see Figs. 2 and 3) and the one lower group is still less advanced. There is no trace of other marginal organs removed. The animal has become almost completely bilaterally symmetrical and shows no indication of returning to a radial pattern.

At this stage the upper, most distal part of the body including the one original tentacle-group and two original anchors and also the two upper reconstituted tentacle-groups was removed by section approximately at AA, Fig. 21, in order to determine whether removal of the original marginal organs and the more distal levels would permit reconstitution to occur at the proximal levels. After this second operation the margin was

still somewhat oblique, but much less so than before. In Fig. 23 the animal is shown in distal aspect as it appeared eight days after the second operation. Seven tentacle-groups and six anchors were present. Three



Figs. 21-26. — Reconstitution after oblique section through umbrella leaving a few of original marginal organs. Figs. 21, 22, two views of same individual 12 days after section; reconstitution of marginal organs retarded at more distal levels and completely inhibited at more proximal levels; AA, approximate level of second operation. Fig. 23, oral view of individual shown in Figs. 21, 22 as it appeared 8 days after second operation; further development of marginal organs has occurred after removal of the part of original margin remaining from first operation. Figs. 24, 25, cases 7 days after section with complete or almost complete inhibition of reconstitution of marginal organs. Fig. 26, a case 7 days after section with marginal reconstitution retarded on the less oblique part of surface of section and completely inhibited on more oblique proximal part.

tentacle-groups and four marginal anchors which had failed entirely to develop on the lower part of the oblique margin after the first operation have now appeared after removal of the distal parts by the second opera-

tion. After the stage of Fig. 23 no further development occurred though the animal lived twelve days longer. During this period reduction in size progressed, tentacles and anchors disappeared and before death the animal was only 3 mm. in length (original length 15 mm.) and resembled Fig. 12 in form, showing merely four marginal elevations without visible differentiation, all anchors having been resorbed.

The interesting result in this case made further experiment desirable, consequently twenty individuals were subjected to similar operation. Three of these died without reconstitution; in the remaining seventeen reconstitution failed occur on the proximal part of the oblique margin in all cases. Figs. 24, 25 and 26 show characteristic cases seven days after section. In the case of Fig. 24 the plane of section was very oblique, that is, the angle between it and the transverse plane was large and two tentacle-groups and three anchors remained. After seven days there was no trace of development of any of the tentacle-groups or anchors removed and no further development occurred in twenty days. In the case of Fig. 25 one tentacle-group and two marginal anchors remained and after seven days only one tentacle-group of those removed was represented by two tentacles in early stage, other groups and anchors being absent. Fig. 26 shows a case in which the plane of section was much less oblique over most of the circumference but very oblique in the most proximal part. Two tentacle-groups and three anchors remained, five groups and three anchors developed on the upper, less oblique part of the margin and none on the lower, more oblique part. The figure shows that the most proximal tentacle-group is less advanced and has fewer tentacles than the others.

TABLE II.

*Reconstitution 7 days after removal of 5-7 tentacle-groups and 5-8 marginal organs by oblique plane of section; 21 individuals of which 3 were dead or dying at time of record.*

Individual number	Number of tentacle-groups removed	Number of anchors removed	7 days	
			Number of tentacle-groups developing	Number of anchors developing
1	6	5	1	0
2	6	6	0	0
3	6	5	0	0
4	6	5	0	0
5	6	5	5	3



6	6	5	2	0
7	6	5	4	2
8	6	5	1	0
9	7	6	1	0
10	6	5	1	0
11	5	5	0	0
12	6	5	0	0
13	6	5	0	0
14	7	6	4	2
15	6	6	1	1
16	7	8	0	0
17	7	6	2	1
18	7	6	3	1
Totals	112	99	25(22.3%)	10(10.1%)

Table II gives numerical data concerning the number of marginal organs which developed within seven days after section in the eighteen individuals which remained alive. The table shows that of a total of 112 tentacle-groups removed only 25 or 22.3 per cent developed and of 99 anchors removed only 10 or 10.1 per cent developed. No further development occurred later without a second operation.

Comparison of Table II with Table I shows that inhibition of development at the more proximal levels of the oblique margin is much greater when a part of the original margin remains (Table II) than when all parts of the margin are removed (Table I). Apparently the fully developed marginal organs are more effective as dominant parts than organs which are developing somewhat more rapidly than others.

Nine of the animals were subjected to a second operation in which the original marginal organs remaining after the first operation were removed as in the case above described (Fig. 21). Eight individuals remained without a second operation as controls to show whether further development occurred. Six days later, however, before records were made, all but three of the animals were dead, apparently from high temperature. The three living were all controls. Observations made from day to day showed that following the second operation additional marginal organs were developing while in the controls no further development occurred. Individual records were not made during this period because it was desired that development should go on for seven or eight days following the second operation before the final records and drawings were made. As regards the effect of the second operation, there is no doubt

that it was followed by development of marginal organs which failed to develop after the first operation but individual records were not obtained because of death.

*Removal of less than half of the margin with 2-4 tentacle-groups and 1-3 anchors.* The purpose of these experiments was to obtain further evidence on the inhibition of reconstitution by marginal organs or regions remaining. From each of ten individuals a part of the margin including 2-4 tentacle-groups and 1-3 anchors was removed. The level to which the cut extended in the proximal direction differed in different cases. The lines XX and YY in Fig. 1 indicate approximately the region removed in certain cases. As these lines indicate, the cut edge of the umbrella is concave to the plane of the original margin and includes different levels of the umbrella.

One of the ten animals died a few days after section, nine lived fifteen days or more. In all cases the development of the parts removed was either much retarded as compared with development at a corresponding level after transverse section, or was completely inhibited.

TABLE III.

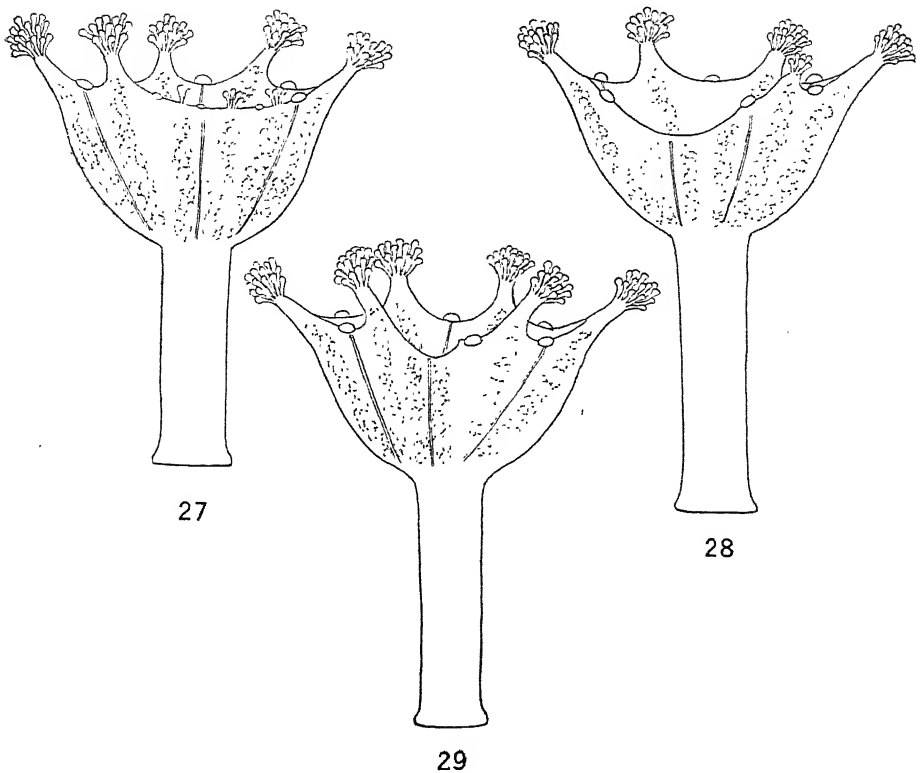
*Reconstitution after removal of 2-4 tentacle-groups and 1-3 anchors. Ten individuals, of which one died before time of record.*

Individual number	Number of	Number of	6 days		15 days	
	tentacle-groups removed	anchors removed	Number of tentacle-groups developing	Number of anchors developing	Number of tentacle-groups developing	Number of anchors developing
1	3	2	3	2	3	2
2	2	1	0	0	0	1
3	3	3	2	1	3	3
4	3	2	0	0	1	0
5	4	2	1	0	1	0
6	2	2	0	0	0	0
7	3	1	1	0	2	1
8	2	2	0	0	0	0
9	3	2	1	0	1	0
Total	25	17	7	3	11	7
Percentages			28	17.6	44	41

Table III gives the numerical data for the nine cases at 6 and 15 days after section. The percentages of tentacle-groups, 28, and of marginal anchors, 17,6 developing 6 days after section are higher than in Table II at 7 days. This is to be expected from the fact that in the present series the section does not extend very far proximally in any case. But these percentages, like those of Table II are much lower than those of Table I at 7 days and this difference, as in the case of Table II is doubtless due to the fact that fully developed, intact marginal organs or regions are more effective in inhibiting reconstitution of others than are organs in early stages of development. Table III shows also that there is considerable development of marginal organs after 6 days. The tentacle-groups increase 16 per cent, the anchors 23.4 per cent, but even at 15 days the percentages of marginal organs reconstituted are much lower than in Table I.

It will be noted from Table III that in two cases, nos. 1 and 3, all of the marginal organs removed developed either in 6 or in 15 days. In both cases, however, development was slow and the marginal organs remained small. In these two animals little more than the margin was removed. Fig. 27 shows No. 3 as it appeared 15 days after section. During this time development of the marginal organs has progressed no further than in 3-5 days after transverse section in the distal or middle umbrellar region. The other case was similar. Five other individuals showed some reconstitution but in all of these some of the marginal organs were permanently absent and those which did develop reached no more advanced stage than those shown in Fig. 27. In general the marginal organs which arise at the more distal levels of the cut are less inhibited than those at more proximal levels.

Two individuals showed no reconstitution of parts of the margin removed, but in one of them, shown in Fig. 28 the cut removed three tentacle-groups and two anchors and also the distal half of another marginal lobe with its tentacle-group. The line of section is indicated approximately by XX in Fig. 1. As shown in Fig. 28 the marginal lobe which was only partly removed did develop a new tentacle-group but none of the other marginal organs removed appeared. In other words reconstitution occurred at the extreme distal level of the middle of the marginal lobe, but not at more proximal levels. This individual is listed as No. 5 in Table III. The other individual which showed no reconstitution (No. 8, Table III) is shown in Fig. 29. Two tentacle-groups and two anchors were removed YY, and after fifteen days the animal was six-rayed and



Figs. 27-29.—Removal of less than one half the margin. Fig. 27, a case in which level of section was only slightly proximal to the margin; 5 days after section; 3 anchors, 3 tentacle-groups removed, all developing but retarded. Fig. 28, a case in which cut extended farther proximal than in Fig. 27; 2 anchors, 3 tentacle-groups removed; no marginal reconstitution 15 days after section. Fig. 29, similar to Fig. 28; 2 anchors, 2 tentacle-groups removed; no marginal reconstitution 15 days after section.

almost perfectly symmetrical in form except for the slight concavity in the margin where the part was removed.

Fifteen days after the first operation most or all of the original marginal organs were removed from the nine animals of this series in order to determine whether reconstitution of the parts removed by the first operation would progress further after the second. All the animals died during the next few days, however, and neither time nor material permitted repetition of the experiment.

*Differential susceptibility.* Since the data on reconstitution indicate the presence of a distal-proximal physiological gradient in the body with high

end in the distal region a few experiments on differential susceptibility were performed in order to determine whether evidence for the existence of a gradient would appear. The agents used were KCN m/100 and m/500, distilled water, tap water and methylene blue. With each agent at least five animals were tested from material collected at different times. Observations on the progress of toxic effect and on recovery were made on animals of different size, the largest and smallest obtainable, 20 mm. or more and 7-8 mm. in length. As regards motor activity and reaction to mechanical stimuli the small animals were distinctly more susceptible than the large and on return to sea water after one hour in KCN m/100 recovered sooner than the large.

As regards the differential susceptibility as indicated by death and disintegration, it was found that in all agents used ectodermal disintegration occurred first in the marginal anchors slightly later in the rounded tips of the tentacles, progressed proximally over the marginal lobes and involved the whole marginal region of the umbrella. On the aboral umbrellar surface the gradient in susceptibility was slight but distal levels were somewhat more susceptible than proximal. The susceptibility of the stalk varied somewhat in different individuals. In animals which were not attached the foot region usually disintegrated slightly earlier than the aboral umbrellar surface and susceptibility decreased from the foot distally for a short distance, but in attached animals the foot appeared somewhat less susceptible than in unattached. The ectoderm of the stalk distal to the foot region usually disintegrated slightly later than the aboral umbrellar surface, but in a few cases slightly earlier. Disintegration usually proceeded in the proximal direction but in a few cases a definite gradient was not observed. The stalk is highly contractile and in animals which are not attached is usually more active than in attached animals. The observations indicate that the variations in susceptibility of the stalk in *Haliclystus*, as in *Hydra* (CHILD and HYMAN, 1919) are probably associated with differences in motor activity.

The contraction of the umbrella in KCN, tap water and distilled water made it difficult to determine the differences in susceptibility on the oral surface of the umbrella, but it was evident that the oral surface was more susceptible than the aboral. Disintegration of the distal region of the manubrium began about the same time as on the umbrellar margin and from these two regions it progressed over the oral surface.

In methylene blue of various concentrations the animals became very deeply stained before toxic effects appeared. The course of disintegration

was essentially the same as in KCN and distilled water. The anchors disintegrated first, then the tentacles and marginal lobes and the distal end of the manubrium and slightly later the foot (all animals used were detached). On the aboral umbrellar surface there was a slight decrease in susceptibility in the proximal direction, as in other agents. The oral umbrellar surface was more susceptible than the aboral, the susceptibility decreasing from the margin and the tip of the manubrium proximally. The susceptibility of the stalk varied somewhat in different individuals, as with other agents.

These observations on susceptibility show that, so far as the ectoderm is concerned the marginal regions and the distal end of the manubrium show the highest susceptibility to the agents used. The oral umbrellar surface is more susceptible than the aboral and both show a decrease in susceptibility in the proximal direction. The susceptibility of the foot is higher than that of the stalk in detached animals and the stalk shows variations, apparently associated with motor activity. In short the distal regions of umbrella and manubrium represent the high end of the chief gradient and another very short gradient in the opposite direction appears in the foot region. As already noted the gradient pattern of *Haliclystus* resembles that of *Hydra* and shows similar variations apparently associated with motor activity.

*Indophenol reaction.* In the five animals used the results as regards differential indophenol formation agreed rather closely with those on susceptibility. The blue color of the indophenol developed most rapidly in the anchors, next in the tentacle tips and distal end of the manubrium and next in the foot region of unattached animals. Coloration progressed proximally on the marginal lobes and manubrium and on the oral surface. No definite gradient was observed on the aboral umbrellar surface and appearance of indophenol in the stalk differed in relative time in different individuals, as did susceptibility.

#### DISCUSSION.

*The character of reconstitution in Haliclystus.* The presence in a form more or less medusoid in character of so great a capacity for extensive and rapid reorganization as occurs in *Haliclystus* is of considerable interest. Moreover, the fact is to be emphasized that reconstitution of an individual from a part occurs to a large extent through transformation or redifferentiation of the part or of certain regions of it rather than through regener-

ation in the stricter sense, that is the outgrowth of embryonic tissue from the cut surface. Whatever the level of section, whether in the umbrella or the stalk, the tentacles and anchors develop directly from the surface of section and other parts removed proximal to the margin develop by the reorganization of regions proximal to the surface of section. For example, in reconstitution of the stalk tentacles and anchors develop directly from the distal surface of section without any visible development of regenerative tissue and the umbrella arises through the transformation of a part of the stalk proximal to the surface of section (Figs. 7, 12, 13). *Haliclystus* is not unique in showing this type of reconstitution. In *Tubularia* and *Corymorpha* there is no outgrowth from the surface of section and in *Planaria* little or nothing but the head regenerates from the anterior surface of section, whatever the body-level. Even in the oligochete annelids, whatever the number of segments removed from the anterior end, only a certain maximum number develop by regeneration (HYMAN, 1916) and others are reconstituted by the reorganization of segments posterior to the surface of section.

In this connection attention may be called to the fact that distal or apical parts, such as the tentacle-groups of *Haliclystus*, the hydranth in *Tubularia* and *Corymorpha*, the head in *Planaria* and the anterior segments in oligochetes may be "out of place" when they first appear. Such reconstitutive development is actually a "heteromorphosis" in LOEB's sense. But, viewed in a different light it is merely an expression of the fact that in reconstitution, as in embryonic development, development begins at the apical or anterior end and progresses basipetally or posteriorly.

*Oral and aboral reconstitution.* Pieces from all levels of the body examined give rise to distal parts on the oral surface of section and by reorganization of the distal region, but in no case has any extensive reconstitution of proximal parts from the aboral surface of section been observed. The only cases in which indications of aboral reorganization into more proximal body-levels were observed were similar to Fig. 15 in which the proximal part of the stalk was removed and the proximal cut end became somewhat flattened but did not develop into a foot and did not become attached. In no case did a stalk develop from a part or from the whole of the umbrella. In a single case the distal half of a stalk developed tentacles and anchors on both oral and aboral ends (Fig. 16). It is possible that with adequate food supply more aboral reconstitution might have occurred, though the extensive distal reconstitution, even in pieces of the stalk indicates that nutrition is not the chief factor concerned.

It appears from the facts that a given body-level may undergo reorganization into a more distal level but that little or no reorganization into a more proximal level occurs. In terms of the physiological gradient these facts mean that at the oral end of the piece which represents the highest gradient level in the piece and is therefore not subordinated to other parts the cells are sufficiently activated by section to grow and undergo reorganization at the expense of other parts of the piece. At the aboral end, however, they represent the low end of the gradient present and section does not activate them sufficiently so that they can grow at the expense of the higher gradient levels distal to them. In short pieces of the stalk with both oral and aboral cut ends there is evidently little gradient and therefore little dominance of one region over another, consequently both ends may be sufficiently activated by section so that both give rise to distal structures (Fig. 16).

As regards the difference in reconstitutive behavior at oral and aboral cut ends *Haliclystus* shows some resemblance to *Tubularia*. In *Tubularia* the aboral end of a piece rarely gives rise to a more proximal body-level or a basal stolon. If it develops at all it almost always gives rise to a hydranth or, if the piece is short, to the more distal levels of a hydranth. In pieces more than 10–12 mm. in length hydranth development usually occurs at both ends of the piece, but in pieces about 10 mm. in length the aboral end very often develops nothing. In these pieces the relations are apparently similar to those in *Haliclystus*, that is the activation by section is not sufficient to permit the cells to undergo reorganization and growth at the expense of parts distal to them. In *Planaria* also short pieces from the anterior region develop no posterior ends, apparently because the cells at the posterior cut surface are not sufficiently activated by section to undergo reorganization and growth at the expense of the very active regions anterior to them. In short anterior pieces of various annelids similar conditions are found. In all of these cases it is apparently the relation between the dominance of more distal or more anterior levels and the effect of section on the cells concerned which determines whether or not reconstitution shall occur.

*The rate and character of reconstitution at different levels of the body.* The results of transverse section at different levels show that although the rate of reconstitution at a particular level differs somewhat in different individuals there is in general a decrease in rate in the proximal or basipetal direction, at least in the umbrella, that is the farther proximal the level of section the slower the development of the marginal organs on



the oral cut end. The difference in rate at different levels also appears in the single individual in reconstitution after oblique section through the umbrella with removal of the whole margin. In these cases the marginal structures of the most distal levels appear earliest and develop and grow most rapidly and the time of appearance becomes later and the rate of development slower as the level becomes more proximal on the oblique surface of section. In the stalk no distinct difference in rate of reconstitution was observed after transverse section at the distal end and the middle. Oblique section of stalks was not made. These data on reconstitution agree with those on differential susceptibility and indophenol reaction in indicating the existence of a physiological gradient in the umbrellar region with its high end distal and in giving no conclusive evidence as regards the stalk.

After transverse section at distal and middle levels of the umbrella reconstitution is almost always complete as regards the number of anchors and tentacle-groups which develop, but in the proximal umbrellar region one or more marginal organs may fail to appear and in the stalk region incomplete reconstitution is still more frequent and the degree of incompleteness greater, only 2-4 radii or adradial being represented in some cases (Fig. 6). It is of interest to note that at these levels the perradial anchors appear before the interradian in those cases in which there is difference in time of appearance and frequently the interradian anchors do not appear at all and only four tentacle-groups develop (Figs. 4, 5, 7, 11-14). The delay in interradian as compared with perradial development is more marked in dilute than in normal sea water. In this earlier development of perradial organs these cases resemble the scyphistoma of the Discomedusae in which the perradial tentacles appear first, the interradian later. The marginal anchors of *Haliclystus* correspond in position to the tentacles of the scyphistoma and the short anchor tentacle is evidently homologous to the scyphistoma tentacle. These cases of reconstitution, as well as the development of the scyphistoma indicate that the perradii represent more primitive features of the organization than the interradii. The adradial marginal lobes which bear the tentacle-groups appear late in reconstitution, even in the most advanced cases and often do not develop at all during the laboratory life of the animals. Doubtless if the animals were fed a greater degree of development of the lobes would occur.

*Dominance and inhibition in reconstitution.* The experiments on oblique section with removal of all marginal organs showed that not only did the rate of reconstitution decrease from distal to proximal levels of the oblique

surface of section, but that some of the marginal organs of the more proximal levels did not appear at all (Fig. 17-20). Moreover, when the oblique section was made in such manner that a few of the original marginal organs remained a still greater degree of inhibition of reconstitution at the more proximal levels of section occurred (Fig. 21, 22, 24-26). At the more distal levels some reconstitution took place, but even there development was much slower and stopped at an earlier stage than after transverse section at the same levels and at the more proximal levels reconstitution did not occur at all. Removal by a second section in transverse plane of the original marginal organs which remained after the first operation was followed by further development of the marginal organs which had already appeared and by appearance of at least some of those previously completely inhibited. The final stage after the second operation was observed in only one case (Figs. 21-23). In all cases the degree of inhibition, as indicated by the rate of development and the size which the organs attain, increases from distal to proximal levels of the oblique surface, that is, the slower the rate of development the more complete the inhibition. At the most proximal levels some of the marginal organs do not appear at all. As the animals undergo reduction in the laboratory resorption of marginal organs progresses from proximal to distal, that is, the more slowly developing and more inhibited organs disappear first. This is evidently only another case of the persistence of organs or regions with a more intense metabolism at the expense of less active regions. Similar relations appear in both the hydroids and planarians.

When only 1-3 adjoining marginal organs are removed the degree of inhibition depends on the body-level concerned. If only the marginal region be removed all organs may be reconstituted, but even in such cases their development is retarded (Fig. 27). If the cut extends farther proximal reconstitution may be completely inhibited (Figs. 28, 29).

These relations between different marginal organs and different body-levels appear to be expressions of physiological dominance associated with the axial gradient. On account of limited time and material it was not possible to determine whether the anchors or more strictly, the nervous tissue associated with them, or the tentacle-groups and marginal lobes were the chief factors in dominance, or whether the marginal region in general is the dominant region. Since the anchors are sense organs as well as organs of attachment and are undoubtedly associated with aggregations of nervous tissue as are the tentaculocysts of other scyphozoa, it seems probable that this organ-complex is the chief factor in dominance,

though the marginal region in general may be concerned.

Perhaps the most interesting fact concerning this dominance is that it is chiefly effective in the distal-proximal direction. After transverse section at the more distal levels the marginal organs of different radii develop at the same rate and there is no indication that they inhibit each other. At the more proximal levels, however, the perradial-organs often develop more rapidly and the interradian organs are apparently retarded in development or do not appear at all. The experimental data indicate that when different marginal organs develop at the same rate they do not inhibit each other, but when they develop at different rates the more rapidly developing may retard or completely inhibit others of slower developmental rate.

*Alteration of axial pattern.* One feature of the cases of reconstitution following oblique section demands special attention. As Figs. 17-22 and 24-26 show, these animals develop and retain a more or less completely bilateral form as regards arrangement and degree of development of the marginal organs. In some cases (Figs. 17, 19, 21, 22, 25, 26) a tentacle-group or an anchor appears on one side of the median plane established by the section and no corresponding organ on the other side, but in other cases, several of which are not figured, the arrangement is completely bilateral (Figs. 18, 20, 24). In all of these cases of oblique reconstitution the most distal region present, whether it be the most distal part of the oblique cut surface (Figs. 17-20) or a part of the original margin (Figs. 21, 22, 24-26), stands in a relation to other parts which is very similar to that of an anterior end or head. It is the high end of a gradient which gives a body-pattern resembling the anteroposterior pattern of cephalized animals. So far as relations of dominance and subordination are concerned, the radial pattern of the marginal organs has been transformed by oblique section into an anteroposterior pattern with more or less complete bilaterality. Moreover, this pattern is not merely a temporary phase of reconstitution but persists as long as the animals live in the laboratory.

This alteration in pattern evidently results from the fact that certain marginal organs or regions are, so to speak, given an advantage over others. This advantage consists either in a higher rate of development at the most distal levels of the oblique surface of section or in intact fully functional condition of a part of the margin with removal of other parts. When all parts of the original margin are removed the most distal region remaining resembles an anterior region in that it develops more

rapidly, and its more intense metabolism determines a dominance over other parts resembling that of an anterior end over other levels of the body. When a part of the original margin remains the effect is essentially the same but the dominance is more effective. In both cases the most distal region represents the highest gradient-level in the piece and its dominance results from that fact. In general dominant organs or regions of an axis inhibit the development of similar organs or regions at other levels of the axis or gradient, at least within a certain distance.

Up to a certain limit the greater the angle between the plane of oblique section and the transverse plane, the further does the pattern depart from the original radial type and approach an anteroposterior, bilateral pattern and the more effective the experimentally established dominant region becomes in inhibiting the development of other marginal organs.

Removal of a few of the marginal organs may result in permanent decrease in the number of these organs through inhibition of development of some or all of the organs removed. This results in alteration of the radial pattern by decrease in number of structurally defined radii.

Section in a plane through the apicobasal axis has resulted in death without reconstitution in all cases, but the results of oblique section lead us to believe that even if the animals lived no reconstitution of marginal organs would occur because the organs already present would completely inhibit any such development.

In connection with these cases of alteration of pattern by oblique section the point is to be emphasized that they are not simply mechanical mutilations, but are real physiological changes in the relations of dominance and subordination from those characteristic of the radial pattern to relations approaching those of anteroposterior pattern. In consequence of this change the marginal developmental pattern becomes heteropolar instead of radial and shows a more or less complete bilaterality along the heteropolar axis.

*The question of the nature of dominance.* The inhibition of less rapidly developing by more rapidly developing parts and the absence of inhibition when similar parts develop at the same rate is characteristic of various other forms as well as of *Haliclystus*. For example, in the stems of the hydroids, *Tubularia* and *Corymorpha* the rate of hydranth reconstitution decreases basipetally and in stem-pieces the development of a hydranth at the oral end retards or inhibits hydranth development at the aboral end except when the piece is very long or very short. In the very long

pieces the aboral end may be beyond the range of dominance by the oral end and therefore not affected by it. But in very short pieces in which the rate of hydranth development at oral and aboral ends is approximately the same the two hydranths develop at the two ends without any indication of inhibition, that is, both develop as rapidly as an oral hydranth on a long piece with oral end at the same level as that of the short piece. Similarly, in very short pieces of *Planaria* heads may develop at the same rate on both ends and without indication of mutual inhibition.

These facts are of interest in connection with the question of the nature of dominance. They indicate on the one hand, that dominance is primarily associated with difference in body-level, that is, of gradient-level, so that parts developing or fully developed at a higher level dominate lower levels to a greater or less degree and retard or inhibit development of certain parts at those levels. On the other hand, such parts, even new hydranths or new heads, developing at the same body-level or gradient-level and so at approximately the same rate, may develop without any indication of inhibition of either, even though they are in close proximity to each other. The interpretation of these facts in terms of chemical substance presents difficulties whether we assume an inhibiting substance produced by the dominant region or a substance necessary for growth and development used up by the dominant region. If there were only the inhibition of a less rapidly developing by a more rapidly developing axis or region such interpretation might be possible, though we have at present no evidence of the existence of such substances in any of the cases mentioned. But in the short pieces of *Tubularia*, *Corymorpha* and *Planaria* and in the reconstitution of *Halicystus* after transverse section the parts concerned are much nearer to each other than in the long pieces or after oblique section but there is no inhibition. In the short pieces of *Tubularia* and *Corymorpha* the whole length may be directly involved in the development of hydranths and the same is true as regards development of heads in very short bipolar pieces of *Planaria*. If an inhibiting substance is produced what becomes of it in such cases? Or if dominance depends on removal of a substance necessary for growth, why is not all such substance used up very early in development in these very short pieces?

If, however, dominance in these cases is primarily transmissive in character no difficulties appear and no special assumptions are necessary in accounting for the various observed facts. The primitive transmission path of the ctenophore plate row exhibits all the phenomena of dominance which we find in development (CHILD, 1917, 1933). One level may

dominate another because the dominant region gives rise to more frequent or stronger impulses than the region dominated. When two impulses transmitted in opposite directions meet they may completely obliterate each other and produce no effect beyond the point of meeting, or one may obliterate the other and continue beyond the point of meeting, but with decreased intensity or strength, as indicated by decreased rate of transmission and decreased amplitude of plate movement. It is probable that mutual obliteration of the opposed impulses occurs when the action currents of the two are approximately equal. Since they are in opposite directions they must obliterate each other and the impulses disappear at the point of meeting. If, however, the action current of one impulse is stronger than that of the other it may obliterate the weaker and still be sufficient to carry on transmission beyond the point of meeting. These various relations of dominance and subordination, mutual obliteration of dominance etc. in the ctenophore plate row constitute a complete functional parallel to the developmental relations of parts. This fact is strong evidence in support of the conclusion that the relations between parts in development are primarily transmissive in character. A region which represents a higher level of physiological activity than regions adjoining it differs from those in electric potential. These potential differences doubtless give rise to electrochemical changes. According to present-day conceptions of protoplasmic transmission the electric currents resulting from differences in potential at different points of a membrane are the essential factors in transmission. A more rapidly developing region must transmit effects more or less continuously to other regions within a certain distance from it. Such a transmitted effect must influence the physiological condition of the parts which it reaches. If a decrement occurs in the course of transmission, as is apparently the case in the primitive protoplasmic path, the transmitted effect may be the chief factor in establishing a physiological gradient. According to this conception one region dominates another because it gives rise to transmitted effects which reach the other. If the other region also gives rise to such effects opposed in direction to those from the dominant region, the latter must be able to obliterate the former and produce effects beyond the point of obliteration, otherwise there is no dominance. For the maintenance of dominance quantitative differences in physiological condition and activity are an essential feature, but the existence of other differences in the parts concerned is of course not excluded. When the transmitted effects from two parts are approximately equal and in opposite directions neither has any effect on the other. This is apparently

the situation in *Haliclystus* after transverse section when the marginal organs all develop at approximately the same rate, and in the short bipolar pieces of *Tubularia*, *Corymorpha* and *Planaria*.

All the known facts of developmental dominance and physiological isolation can be interpreted without difficulty in terms of transmitted effects of physiological activity to less active regions. As already noted, the developmental relations of parts are completely paralleled by functional relations in primitive transmission paths, such as the ctenophore plate row. Moreover, in organisms in which a nervous system differentiates the region which was dominant in early development becomes the region of development of the chief aggregation of nervous tissue and of nervous dominance. In a few cases the polar gradient changes early in the course of development and the definitive dominant region is not the region of primary dominance. These cases, however, do not affect the general conception for they are merely case in which a new axis, a new gradient, appears early in development, either as the result of budding or of some other condition. With increase in the differentiation of organs in the course of ontogeny and of evolution the metabolism of particular organs gives rise to specific substances and the transport of these substances to other parts of the body becomes an increasingly important factor in correlation. This, however, is evidently a secondary form of correlation and dominance resulting from the specific differentiation of different organs and it is apparently very different in character from the relations of dominance and subordination which we find in the simpler organisms and in early stages of development as well as in reconstitution.

#### SUMMARY.

1. After transverse section at various levels of the umbrella, at the junction between stalk and umbrella and at various levels of the stalk reconstitution of distal parts occurs rapidly in the proximal piece.
2. In reconstitution tentacle-groups and marginal anchors develop on the surface of section and other parts removed are formed by reorganization of regions proximal to the level of section. In reconstitution of the stalk or a part of it the distal portion of the stalk or piece undergoes transformation into the umbrella.
3. In distal pieces possessing marginal organs almost no reconstitution of proximal parts removed or reorganization beyond healing of the wound occurs.

4. A single case of bipolarity was observed in the distal half of a stalk: this piece developed a few marginal organs on both oral and aboral ends.

5. In general the rate of reconstitution decreases as the level of section becomes more proximal but individual differences in rate at a given body-level occur. These seem to be most marked in animals of different size.

6. At levels of section in the proximal umbrellar region, at the junction of umbrella and stalk and in the stalk, the perradial anchors often develop before the interradian and in some cases the interradian anchors do not appear at all.

7. After oblique section through the umbrella with removal of all parts of the original margin the rate of reconstitution decreases in the proximal direction on the oblique surface of section and reconstitution at the more proximal levels is more or less inhibited, apparently by the distal levels. At the most proximal levels of the oblique surface this inhibition may be complete.

8. When oblique section is made in such manner as to leave intact 1-3 original tentacle-groups and marginal anchors reconstitution of marginal organs at more proximal levels is even more inhibited and in some cases none of the marginal organs removed develop.

9. After a second operation removing the parts of the original margin which remained from the first operation, marginal organs previously inhibited may develop.

10. In the course of reduction during laboratory life the more inhibited organs on oblique surfaces of section disappear first, those of the distal, dominant region last.

11. When 1-3 tentacle-groups and anchors are removed the result depends on the depth of section. If only the marginal region is removed all marginal organs removed may develop, but their development is retarded and they remain small. When the piece removed includes more proximal levels the degree of inhibition of development is greater and there is often complete inhibition.

12. In general marginal organs which develop at the same body-level and at approximately the same rate do not inhibit each other but when the rates of development of different organs are different, e.g., when they develop at different body-levels after oblique section, the more rapidly developing regions or those which are fully developed inhibit the less rapidly developing. These relations are expressions of physiological domi-



nance and it is shown that they do not differ essentially from conditions observed in other forms.

13. The change in the relations of parts resulting from oblique section determines the development of a heteropolar, instead of a radial pattern of arrangement of marginal organs with the most distal, dominant region representing anterior end or head and with an approach to, or attainment of bilaterality.

14. Data on differential susceptibility and on the indophenol reaction, as well as those on reconstitution at different levels indicate the presence of a physiological gradient, at least in the umbrellar region. The distal margin of the umbrella and the distal end of the manubrium represent the high end of this gradient.

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# ON THE ORGANIC IODINE IN *LAMINARIA OCHOTENSIS* MIYABE WITH ESPECIAL REFERENCE TO PROTEIN IODINE, AND SEARCH FOR DIIODOTYROSINE.

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Since it seems probable that the eating of *Laminaria* is mainly responsible for the almost total absence of goiter in Japan, the chemical combination of the iodine is of interest.

## I) Protein Iodine.

The material used in our experiment was *Laminaria ochotensis* Miyabe. Commercial material (Kombu) was obtained, dried, ground, and powdered. For the determination of alcohol-soluble and insoluble iodine, the powdered sample containing 0.23% of total iodine was extracted in 95% alcohol for 8 hours under a reflex condenser. The presense of iodine in both the extract and the remainder from alcohol was determined by McCLENDON's method.

To determine the protein iodine in the *Laminaria* the following method was employed. The dried powdered material was immersed for 1 hour in 100 volumes of 0.5% sodium carbonate, the mixture stirred continuously with a glass rod. The solution containing many substances including the viscous carbohydrate (alginic acid) was filtered off. The remaining impure protein was washed well with distilled water, dried and analysed for the determination of iodine. McCLENDON's method was also employed in this case.

The results obtained are given in Table I.

As will be seen in Table 1, the amount of iodine contained in the remainder from alcohol extract is estimated about 0.02%, corresponding to about 5.7% of the total iodine and that in the protein is about 0.03%, corresponding to about 4.7% of the total iodine. Therefore, the alcohol-insoluble iodine in the *Laminaria ochotensis* is in protein for the most part (82%), and only about 1% of the total amount of iodine or 18% is non-protein.

TABLE I.  
Partition of iodine in *Laminaria*.

Sample in gm.	Form of iodine	Iodine content in gm.	Percentage of iodine	Percentage of iodine against the total iodine
1.5 (Powder)	Total iodine	0.00345095	0.230073	
0.9424 (Remainder from alc. extract.)	Alcohol-insoluble	0.00019692	0.020896	5.735021
0.4500 (Protein)	Protein iodine	0.00016168	0.032454	4.714328
0.4924 (Non-Protein residue)	Non-Protein alcohol-insoluble	0.00003524	0.007158	1.020693
0.6000 (Alc. extract)	Alcohol-soluble	0.00310688	0.501111	94.264979

TSUKAMOTO and FURUKAWA ('18) analysed the commercial *Laminaria* and found that about 95% of the total iodine is inorganic and about 5% is organic. This result almost agrees with my own data for alcohol-insoluble iodine. OKUDA and ETO ('20 and '25) examined several kinds of fresh seaweed, *Eisenia arborea* Aresch. f. *bicyclis* YENDO, *Ecklonia cava* KJELLM, *Sargassum enerve* AG. and *Turbinaria fusiformis* YENDO and reported that 95% of the total iodine is organic and only 5% of it is inorganic, but much of this organic iodine is alcohol-soluble. They stated, however, in the same paper, that about 5% of the total iodine is insoluble in water. They found that drying while allowing autolysis and fermentation to proceed as ordinarily done commercially, changed most of the organic iodine to inorganic.

## II) Search for the Presence of Diiodotyrosine in *Laminaria ochotensis*.

HARRINGTON and RANDALL ('29) and FOSTER ('29) have reported the isolation of diiodotyrosine from the thyroid gland. DRECHSEL examined *Gorgornia cavolinii* (the sea fan) and found iodogorgonic acid (diiodotyrosine) in the protein, gorgonin. HUNDESHAGE analysed sponge and found iodospongin yielding diiodotyrosine on hydrolysis. Thus diiodotyrosine is a substance which is found in a gland present in all vertebrates and has been found in scleroproteins in lower invertebrates living in sea water. As far as we know, however, there is no one who has studied diiodotyrosine in vegetables.

*Laminaria ochotensis* contains a large amount of protein iodine as

already mentioned. Moreover, it is generally thought that there is a similarity between the *Laminaria* and the lower invertebrates in their ability of making organic iodine from inorganic iodine (dissolved in seawater). Therefore, an attempt was made in the present investigation to examine the question as to whether the isolation of diiodotyrosine from the protein in the *Laminaria* is possible.

The protein containing 0.032% of iodine was obtained from *Laminaria ochotensis* by the method already described. The presence of diiodotyrosine was investigated by the method employed in the isolation of diiodotyrosine from the thyroid gland by FOSTER ('29). Two samples were examined, the first consisting approximately of 100 gm.; the second 150 gm. In none of these samples were crystals of diiodotyrosine detected, but the supernatant fluid and the precipitate obtained in the final step of the series of the technique employed were dried and used for the determination of iodine by McCLENDON's method, with the following results (Table II).

TABLE II.

No. of experiment	Material	Weight of material in gm.	Iodine content in mg.	Percentage of iodine
1st. (100 gm. of protein)	Dried supernatant fluid	4.200	1.52100	0.03621
	Dried precipitate	0.039	0.01759	0.04500
2nd. (150 gm. of protein)	Dried supernatant fluid	4.239	0.04293	0.00100
	Dried precipitate	0.050	Trace	—

As will be seen in Table II, both the supernatant fluid and the precipitate contain a small amount of iodine, suggesting that diiodotyrosine may exist in the protein from *Laminaria ochotensis*, though it was not isolated as crystals. The handling of an enormously large amount of protein would be required in order to settle the question. Perhaps it would be better to use the Kombu-Powder instead of the protein fraction.

## SUMMARY.

1) In *Laminaria ochotensis*, about 6 per cent of the total iodine is alcohol-insoluble, 5 per cent being protein iodine and 1 per cent is non-protein iodine.

2) Diiodotyrosine may exist in the protein from *Laminaria ochotensis*, but it was not isolated as crystals from samples of 100 gm. to 150 gm. of protein.

Before leaving the subject, I wish to express my sincere thanks to Dr. J. F. McCLENDON for his valuable guidance and criticism throughout this work.

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# A STUDY OF THE RESPIRATORY CONDITIONS IN SEA WATER AQUARIUM.<sup>1)</sup>

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## INTRODUCTION.

In keeping fishes in an aquarium the most vital essential is the physiology of respiration. At the same time, because the gas condition is good it does not necessarily follow that other physiological conditions are favourable. For all that primary importance must be attached to the study of respiratory conditions in an aquarium. Unlike most respiratory studies in which the fishes are confined in a closed water system aquaria are usually kept open, with result that the oxygen used up by fishes is constantly being replenished from the open surface of the aquarium as well as from the inflowing water. On the other hand the  $\text{CO}_2$  exhaled by the fishes is correspondingly expelled by the jets of bubbles. The evasion and invasion of  $\text{CO}_2$  and  $\text{O}_2$  from and to the surface of the water can not be taken into account owing to the practical difficulty of their determination. The results thus obtained are, however, of importance in considering the actual conditions in which the fishes are living normally. The present experiment has been conducted with this end in view, stress being laid on the observation of the change of the pH and oxygen content of water.

### 1) CIRCULATORY SYSTEM OF AQUARIUM WATER.

In the aquaria at our station (Asamushi) the fresh sea water which is drawn from the sea is drained off after once passing the exhibition tanks. The water is thus never used twice. Hence no closed circulatory facilities are provided. This prodigal use of fresh sea water is due to the unique location of the station where an unlimited supply of clear water is easily available from the adjacent coast free from turbidity all the year round. The sea water is drawn from one side of a small spit and ejected on the other side after passing through the aquarium.

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<sup>1)</sup> Contributions from the Marine Biological Station, Asamushi, Aomori-Ken, No. 100.

In the aquaria of the Plymouth Laboratory and the Zoological Society of London etc., where the sea water is supplied at intervals, circumstances make it necessary to use the same water repeatedly for a considerable time. Various authorities have therefore speculated as to whether any radical changes might be found in water thus used. ATKINS (1922, 1931), STOWELL (1925), BROWN (1929), have studied aquarium water in this respect. As previously mentioned the water in our laboratory passes through the fish tank once only, so that no notable changes might be expected other than the gaseous content and pH which change only to an extent compatible with the respiration of the fishes.

The high-level reservoir of sea water at Asamushi has a capacity of about 25 cubic meters. From this tank water flows continually through the aquarium and laboratory. It requires about 2 hours to fill up the reservoir by means of a 4 H. P. internal combustion pump. In summer when the laboratory and aquarium are at their maximum activity the reservoir is filled 3 or 4 times a day. There are 23 exhibition tanks. Of these, the eleven main tanks, on which the present observations are based, are each of 2600 litres in capacity except one which contains rather more than double that amount.

The feed to each tank varies between 1 and 2 litres per minute. The regulation of the flow of water is irregular owing to the fact that when the reservoir is full the water supply increases on account of the increased "head". When the reservoir becomes nearly empty the pressure and flow are reduced by about 50%. It will be seen from Table I that this variation of flow has a significant bearing upon the respiratory condition of the water in the tank.

The aeration is made gravitationary by inserting into the rubber joint of the inflow tubing a thin glass tube terminating in a point. By this process the inflow water carries with it a mist of air-bubbles which slowly rise upwards from the nozzle of the glass pipes. Except the largest tank (No. 6), which duplicate inflow and overflow tubes respectively, each of all the tanks are provided with one of these tubes.

## 2) FISH TANK.

Observations were made on eleven tanks. The large one (No. 6) measured about 3 meters in length, 1.4 meters in breadth, and 1.8 meters in depth, while the remainder were 1.35 meters in length but in other respects the same as the large one. Though each tank is named by its chief occupant they frequently contained other species.

- Tank No. 1, "Conger". 11 congers (*Astroconger myriaster* BREV.) and leather-fish (*Canthierines modestus* GUNTHER). Of the former 5 were about 75 cm in length, the remainder about 20-23 cm. Body length of two of the leather-fish, 33 and 17 cm.
- Tank No. 2, "Sebastodes". 13 Sebastodes (*S. schlegeli* HILG.), a bass-like fish, of which 10 were longer than 32 cm, the smallest being about 23 cm. Three sea-cucumbers (*Sticopus japonicus* SELENKA), about 20 cm were also present.
- Tank No. 3, "Sebastodes". 28 Sebastodes (*S. guntheri* J. & S.), a bass-like fish, all nearly the same size viz. 24 cm. Two swimming crabs (*Portunus trituberculatus* MIERS) of moderate size inhabited the bottom.
- Tank No. 4, "Hexagrammos". *Hexagrammos otakii* J. & S., about 47 cm in length. Also 2 small *Sebastodes schlegeli* HILG. about 18 cm. each.
- Tank No. 5, "Mackerel". This tank contained several species comprising 8 mackerel, about 20-25 cm. eleven horse-mackerel (*Trachurus japonicus* T. & S.) about 15 cm., seven small yellow-tail (*Seriola aureovittata* T. & S.) (15-20 cm), three *Sebastodes guntheri* J. & S. about 13 cm. Three flat fish (*Platichthys stellatus* PALLAS), inhabited the bottom.
- Tank No. 6, "Porgy". This is the large tank. It contained 14 Porgies (*Pagrosomus Major* T. & S.) of various sizes, the four largest being about 40 cm. in length. Besides Porgies there were three dogfishes (*Halaelurus torazame* TANAKA) and skate (*Raja meerdervoosti* BLEEKER), all of moderate size.
- Tank No. 7, "Physiculus". 38 *Physiculus japonica* HILG. about 22 cm. Several sea-squirts (*Cynthia roretzi* v. DRASCHE) and starfishes (*Asterias* sp.) were included.
- Tank No. 8, "Yellow-tail". Sebastichthys (*S. mitsukurii* CRAMER), ranging in length 22 to 38 cm.
- Tank No. 9, "Yellow-tail". 53 yellow-tails (*Seriola aureovittata* T. & S.). All being young their average length was barely 20 cm.
- Tank No. 10, "Myxocephalus". 7 *Myxocephalus* (*M. raninus* J. & S.) about 25 cm. and 3 Auma (*A. emmion* J. & S.) about 34 cm. in length.
- Tank No. 11, "Globe-fish". This tank contained 18 Globe-fish (*Sphaeroides rubripes* T. & S.), 15 and 33 cm in length.

### 3) PHYSICAL PROPERTIES OF AQUARIUM WATER.

#### a) Water temperature.

Early in May, when the aquarium is opened annually the temperature of the aquarium sea water is usually a little lower than 10°C. It was, for instance, 9.7°C on May 3, 1932, the fresh water aquarium being 9.1°C on the same date. Thenceforth it gradually rises, until it reaches its highest, about 25°C at the warmest season, after which the temperature gradually falls. When the aquarium closes at the end of October the temperature is usually about 16°C. The following table gives the temperature of both the sea-water and fresh-water aquarium compared with the temperature of sea water at the laboratory's pier.



(1932)	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Sea-water aqu.	—	—	—	—	11.3	14.1	17.9	24.1	19.1	16.5	—	—
Fresh-water aqu.	—	—	—	—	10.5	12.6	15.7	20.8	17.6	14.4	—	—
At pier	7.34	6.29	6.81	8.77	12.78	16.27	19.80	23.40	21.60	17.60	13.30	8.83

It will be seen from this table that the monthly mean temperature of a sea water aquarium slightly differs from that observed at the pier. Though the temperature of sea water usually exhibits a diurnal fluctuation this has not been observed in our aquarium. As the aquarium water is drawn from the sea irrespective of the time of day, while some of the previous day's water remains, the diurnal fluctuation in the tanks is very irregular.

As it may serve as a reference for the management of aquarium, a brief account of the diurnal fluctuation of water temperature will be given. According to observations, made three times a day, during 1927-1930, at our pier, the monthly mean of the diurnal fluctuation of water temperature is largest in April and smallest in December, see the following table. The maximum diurnal fluctuation in April sometimes exceeds 5°C, e.g. 5.9° on Apr. 9, 1927. Such fluctuation are far greater than have been recorded in the open sea where the fluctuation is said to be seldom more than 1°C.

(Monthly mean of diurnal fluctuation observed during 1927-1930).

Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Fluctuation	0.5	1.0	1.3	2.1	1.9	1.5	1.3	0.9	0.8	1.0	0.5	0.4

On examining the results of the daily observation of the temperature of the water in the aquarium we found that there was no temperature change other than the seasonal one, and that the aquarium temperature always closely approximated that of the sea.

The temperature of the aquarium water at the time of the experiments was 22.6° and 23.0°C in Exp. I and Exp. II respectively.

TABLE I.

No. of Aqu.	Water temp.		Change of water flow per m. in cc.			Change of pH						Change of O <sub>2</sub>					
						Exp. I			Exp. II			Exp. I			Exp. II		
	Exp. I	Exp. II	Exp. I	Exp. II	Diff. in %	Inflow	Overfl.	Diff.	Inflow	Overfl.	Diff.	Inflow	Overfl.	Diff.	Inflow	Overfl.	Diff.
1	22.6	23.0	1,500	622	57%	8.30	7.95	0.35	8.30	7.90	0.40	4.73	3.87	0.87	4.46	2.96	1.50
2	"	"	2,307	1,200	53%	"	7.90	0.40	"	7.82	0.48	4.87	3.81	1.06	4.49	3.79	0.70
3	"	"	1,622	800	49%	"	8.05	0.25	"	7.95	0.35	5.02	3.96	1.06	5.11	3.56	1.56
4	"	"	2,500	588	24%	"	8.10	0.20	"	7.95	0.35	4.77	4.59	0.18	5.04	3.87	1.17
5	"	"	2,727	1,364	50%	"	7.90	0.30	"	7.80	0.50	4.75	3.23	1.53	4.50	2.10	2.40
6 <sup>a)</sup>	"	"	1,500	1,000	67%	"			"			4.82		4.72			
6 <sup>b)</sup>	"	"	1,023	760	74%	"	7.70	0.60	"	7.60	0.70	4.94	2.72	2.22	4.24	2.23	2.25
7	"	"	1,041	818	79%	"	7.90	0.40	"	7.85	0.45	4.87	4.11	0.76	4.31	3.87	0.43
8	"	"	1,445	500	35%	"	7.92	0.33	"	7.70	0.60	4.96	4.00	0.97	5.08	3.10	1.99
9	"	"	2,142	1,122	55%	"	8.00	0.30	"	7.90	0.40	4.80	3.56	1.24	4.85	3.15	1.70
10	"	"	1,818	750	41%	"	8.05	0.25	"	7.80	0.50	4.75	4.43	0.32	4.58	3.41	1.17
11	"	"	1,304	483	37%	"	8.10	0.20	"	7.80	0.50	4.59	3.91	0.67	4.41	3.42	0.99
Mean	22.6	23.0	1,744	921	52%	8.30	7.96	0.34	8.30	7.82	0.48	4.82	3.84	0.99	4.65	3.31	1.44

b) Specific gravity ( $S_4^{17}$ ) of sea water.

The aquarium water of the Asamushi Station has a mean annual specific gravity of about 24.00. This value is somewhat low as compared with that of the adjacent sea, e.g. Tsugaru Strait, where it is generally 25.50 or thereabouts. This is due to the fact that Mutsu Bay, in which the Asamushi Station is located communicates by a narrow entrance with the Tsugaru Strait. The sea water which enters from the strait is therefore, permanently diluted by the constant inflow of fresh water from the rivers which drain into it. HATAI and KOKUBO (1928) confirmed the fact that the specific gravity of the surface water of Mutsu Bay shows a remarkable seasonal change as indicated in the observations of 1926. Observations during the succeeding four years demonstrated that their conclusions were fairly consistent with the data obtained during the following four years. The results of the five years observation, including 1926, are tabulated below. From the table it can be seen that every year the specific gravity is largest in September and smallest in April, the monthly mean ranging from 21.40(1929) to 25.24(1928). The highest and lowest values so far observed are 12.87(Apr. 30, 1927) and 26.23(Sept. 7, 1928) respectively both probably being exceptional cases.

TABLE II.

Month \ Year	1926	1927	1928	1929	1930	Mean
January	24.82	23.86	23.83	23.99	23.81	24.06
February	24.61	24.01	24.10	24.29	24.53	24.31
March	24.60	23.39	23.71	23.36	23.93	23.80
April	21.71	20.45	21.82	21.40	23.43	21.76
May	22.90	21.94	22.30	22.15	23.95	22.65
June	24.16	23.59	23.51	23.06	23.79	23.62
July	24.82	23.34	23.91	23.97	23.93	23.99
August	24.95	24.33	25.11	25.15	24.87	24.88
September	25.05	24.31	25.24	25.16	24.69	24.89
October	24.94	24.63	25.09	25.00	25.27	24.99
November	24.53	24.09	24.15	24.41	24.38	24.31
December	24.40	23.98	24.33	24.16	24.31	24.24
Mean	—	—	—	—	—	23.91

Monthly mean of the specific gravity ( $S_{15}^t$ ) of sea water observed at our pier during 1926-1930. Calculated from the observations made three times every day at 7.00 am., noon, and 4.00 p.m.

It will be seen from the table that during the period, February to July, the specific gravity shows such remarkable changes that these six months may be termed the "variable period". Contrasted with this the six months from August to January might be called the "constant period" owing to the definitely fewer changes occurring during this season.

No doubt the rapid decrease observed during February and March might be due to the very large quantity of snow water brought down by the rivers into the bay. With the seasonal diminution of snow water the specific gravity rises rapidly from early April on and recovers its normal value in mid-July. A further rise of specific gravity from this until October may be attributed to the predominance of the warm current which comes from the Tsugaru Strait. This is also indicated by the fact that tropical plankton which commence to appear in August reaches its maximum in mid-October. After October the specific gravity, due to the decline of the warm current, gradually decreases, but the change during the three months, November to January, is relatively slight. It must be remembered that the observed seasonal changes refer only to the surface water. With deeper water the variation may differ very considerably.

According to the above mentioned results it will be noticed that the

aquarium is opened every year after the maximum fluctuation of salinity has passed, though in early May the specific gravity is not only much below normal but is also unsteady. These conditions may not be so extreme as to affect the physiology of aquarium organisms as the specific gravity returns gradually to normal from mid-May onward. From then until the aquarium is closed at the end of October no special change has been noticed.

#### 4) EXPERIMENT.

For the purpose of observing the gas conditions in aquaria the pH and oxygen content of the inflow- and outflow waters was determined in each tank and compared. As already mentioned the speed of the circulating water varies with head in the storage reservoir. Two separate determinations have therefore been made, viz. Exp. I when the reservoir is quite full, Exp. II when the latter is almost empty. By this means the oxygen consumption and carbon dioxide output of fishes under different gas tensions in the same tank may be measured. The determination of oxygen content was made by the ordinary WINKLER's method, and the pH was measured by using the indicator and colour standard of thymol blue and cresol red regarding the salt error to be 0.2 in both cases.

##### a) Change of oxygen content.

**OXYGEN CONTENT OF WATER.** The oxygen content of the sea water close to the entrance of the intake pipe was 4.33 cc per litre, showing a percentage saturation of about 91%. On entering the fish tank the oxygen content of this water increases slightly by aeration. This increase varies for each tank due probably to the amount of air inhaled from the aerating fine glass tube. For instance in Tanks No. 1, No. 7 and No. 11 no increase was observed, although the average increase was 4.82 cc per litre at the beginning and to 4.66 at the end, i.e. 93% and 90% respectively. It is evident that the aeration not only increases the tension of oxygen in the fish tank but it also increases the percentage saturation of freshly entering sea water.

As can be seen from Table III, the oxygen content of the freshly entering sea water decreased about 4% in percentage saturation during its 6 hour storage. This may be due in part to the oxygen consumption of plankton organisms whose photosynthesis is inhibited due to the lack of light in the reservoir, though it may, to some extent, be ascribed to

the lessened aeration due to the decreased pressure in the reservoir tank.

**DECREASE OF OXYGEN.** In the fish tank the oxygen content decreases because of the breathing of the fishes. The rate of decrease is proportionate to the flow of water and to the number of fishes etc. As already mentioned the flow always decreased during the experiment partly due to the lowering of head in the reservoir and partly to the choke of the inflow pipe.

The rate of decrease varied according to tank, but on an average an initial flow of 1.744 litres per minute (Exp. I) decreased to 0.921 litres per minute, i.e. 52% in about 6 hours (Table I).

**Experiment I.** In Exp. I oxygen decrease of each tank ranged from 0.18 cc (Tank No. 4) to 2.22 cc (Tank No. 6), showing a mean value of 0.91 cc per litre. The low value of Tank No. 4 was due both to the comparatively high rate of flow and to the lesser quantity of fishes in the tank. On the contrary, the oxygen consumption of Tank No. 6 is due to the dense crowd of fishes in this tank which was inhabited by large Porgies and the deficiency of oxygen was apparently reflected by their breathing which seemed more or less laborious.

When the average value is taken it may be correct to state that at 22.6°C. the oxygen consumption per minute is 1.73 cc per tank when the incoming sea water is delivered at the rate of 1.744 litres and the fishes are kept in the usual numbers. Extreme cases show much difference, the largest consumption being, (Tank No. 6), 5.55 cc per min. and the smallest (Tank No. 4), 0.45 cc per min. From these results it can be seen that if the flow of water to Tank No. 6 were reduced to only 1 litre per minute the fishes would have to absorb the oxygen until its tension becomes 0. This might prove impossible physiologically as the dissociation of oxygen from the blood of fishes may safely be made when the oxygen tension is kept higher than one third of the normal. In the case of Tank No. 6, therefore, roughly more than 1.5 litres of water should be supplied every minute in order to keep the fish normally.

When expressed in terms of percentage saturation the decrease of oxygen in each tank may be tabulated as follows.

TABLE III.

Decrease of oxygen content shown in percentage.

No. of tank	Exp. I			Exp. II		
	Percent. sat. of inflow water (%)	Percent. sat. of overflow water (%)	Consumption of O <sub>2</sub> in % for initial content	Percent. sat. of inflow water (%)	Percent. sat. of overflow water (%)	Consumption of O <sub>2</sub> in % for initial content
1	91	74	19	86	57	34
2	94	73	22	86	73	16
3	98	76	21	98	86	30
4	92	88	4	97	74	23
5	91	62	32	87	40	53
6	94	52	46	86	43	50
7	94	79	16	83	75	10
8	95	77	20	93	61	39
9	92	89	26	88	66	35
10	91	75	5	85	66	26
11	98	85	15	90	60	22
Mean	93	75	21	89	63	31

The above table shows that the lowest percentage saturation of outflow water was 52%.

Experiment II. Experiment II was carried out 6 hours after Exp. I, when the reservoir is almost empty. Taking the average of all the tanks the flow had decreased by about 48%. The rate of decrease of flow varies according to the tank, ranging between 24% and 79% of the initial flow.

It might be expected that the decrease of oxygen per minute per litre of water would increase inversely with the rate of flow, assuming that the rate of oxygen consumption of fishes remains constant through the range of oxygen tension under discussion. That this was actually the case can be seen by the last three columns of Table I. According to the mean value the decrease of oxygen per litre was 1.44 cc, i.e. about 1.5 times of the initial rate of decrease. Therefore it follows that when the rate of flow becomes 1/1.9 of the initial flow the oxygen decrease becomes 1.5 times as much as the initial rate of decrease.

Calculation from the mean value (1.44-0.92) shows that the oxygen consumption per minute is 1.33 cc per tank. From a comparison of this value to that of the Exp. I it will be observed that the consumption in Exp. II is much less than in Exp. I. Though HALL (1929) found recently that in some fish the rate of oxygen consumption depends upon the oxygen tension of the water, the decrease in this case can not be compared with HALL's result. In the present experiments some considerable amount of oxygen may have been drawn from the open surface of the tank. Accord-

ingly it appears that the oxygen consumption may actually have been almost the same as in Exp. I.

Summing up the above result it may be stated that under normal summer conditions at Asamushi the decrease of oxygen content per minute in the feeding water in each tank ranges from 1.33 to 1.73 cc on an average in the eleven tanks. In a very crowded tank (Tank No. 5, Exp. II) the decrease may be as great as 6.48 cc per minute. As the rate of flow slows down the decrease of oxygen per minute increases, but if the speed of the flow is taken into account the total decrease seems to remain almost constant.

#### b) Change of pH.

**pH OF SEA WATER.** The pH of fresh sea water in shore near the orifice of the suction pipe was 8.30 before it was drawn into the reservoir. It showed no change during its six hour storage. According to my previous work (1932) the annual mean pH of the off-shore water was found to be 8.21 in 1929, and 8.19 in 1930. The highest value found was pH 8.30, on Sept. 19th, 1929, when the diatoms vegetated abundantly. In the Asamushi aquarium water which is drawn up from the littoral zone where algae flourishes, a pH of 8.3 has frequently been observed. As already mentioned the oxygen content of the sea water decreased slightly during storage, while the pH showed no change in spite of the fact that the respiration of plankton organisms probably evolved carbon dioxide due to lack of sunlight.

The excess base on which the pH of sea water is dependent was 24.3 cc as measured by McCLENDON's method (1917) on fresh sea water. According to BROWN (1929) the excess base of aquarium water which was 23 cc at first decreased to 16 cc after being in use for one month. In the present experiment this measurement was not made as the water was in a state of constant replenishment so that no appreciable decrease was anticipated.

**DECREASE OF pH.** According to ATKINS (1922) the reservoir of the Plymouth Aquarium is always at a pH of about 7.6, a decrease of pH 0.6-0.7 as compared with fresh sea water. In the fish tanks, however, the pH usually varied between 7.50 and 7.27, and in some abnormal cases, it was as low as pH 7.2-7.05. STOWELL (1925) observed the pH of the tank water of the Zoological Society's Aquarium, London, and states that the pH of the outflow varied between 7.70 and 7.85, while the pH of the sheltering tank remained at 7.85. In STOWELL's case the decrease of pH

is very slight either because of the relatively small number of fishes in the tank or because of the rapid change of the tank water. BROWN (1929) also studied the water of the aquarium of Zoological Society of London and found that the pH of the tank water ranged from 7.02 to 8.22. Among the tanks which he observed the dogfish tank which seems to have been the most crowded showed a mean pH of 7.25.

Research indicates that the pH of the outflow water of the Asamushi aquarium is much higher than those of the above aquaria. This is doubtless due to the rich supply of fresh sea water whose pH is much higher than those of water kept for a long time in storage.

Experiment I. The oxygen consumed by fishes in a tank is used for oxydation in the fish's body and is secreted in the water as carbon dioxide. Assuming the R. Q. of fishes to be 0.8 the 100 cc of oxygen absorbed by the fishes will produce 80 cc of carbon dioxide. The  $\text{CO}_2$  thus evolved greatly increases the tension of this gas in the tank water so that the acid base equilibrium of the sea water is shifted to the acid side, thus decreasing the pH of the water.

In Exp. I (Table I) the pH of inflow water which was 8.3 decreased to 7.96 taking the mean value of eleven tanks, showing a mean difference of pH 0.34 between inflow and outflow water. The extent of change varied with the tank, the range being from pH 7.70 in Tank No. 6 to pH 8.10 in Tank No. 11. In cases where the change was extreme the decrease of pH attained 0.60. This change is reflected by the fact that the tank in question was densely crowded. Comparing the decrease of pH with that of oxygen one will note that in tank No. 4 where the oxygen decrease is least the pH decrease is also and vice versa (e.g. Tank No. 6). Although the order of the tanks dose not strictly coincide with the decrease of pH and oxygen, both values are nevertheless approximately proportional.

Experiment II. As previously noted the reduction of the flow resulted in a rapid decrease of oxygen content. Comparison of Exp. I with Exp. II suggests similar relation in respect to the change of pH values, showing that the slower the flow the lower the pH becomes. In Exp. II, as in the case of oxygen, the pH of the inflow water was 8.30, showing no change in the course of 6 hours. The outflow water, however, showed a much lower pH than that in Exp. I, giving a mean value of 7.82. The difference, therefore, between inflow and outflow water turns out to be 0.46. The range of the change of pH was from 7.6 to 7.9, showing a lower range than that in Exp. I. As in Exp. I the lowest and highest



pH were found respectively in Tanks No. 6 and No. 4.

The mean decrease of pH in Exp. II (pH 0.48) is less by pH 0.14 than in Exp. I. The greater decrease is due doubtlessly to the reduced speed of inflow water in Exp. II, as will be surmised from the greater consumption of oxygen in Exp. II than in Exp. I.

ATKINS (1922) shows that whilst the reservoir was at pH 7.60 the pH of the tank water ranged from 7.57–7.27 in one case to 7.72–7.45 in another case. Assuming the mean value (pH 7.48) of these four observations to represent the pH of the tank water it will be seen that the pH difference between reservoir- and tank water is pH 0.17. In STOWELL's (1925) case the pH of the outflow (pH 7.78) was lower than that in the sheltering tank by pH 0.07. BROWN's (1929) results shows that in the dogfish tank, which seems somewhat crowded, the pH was 7.25 taking an average of nine observations and showed a difference of pH 0.89 in comparison to the pH of new water which showed pH 8.14 as the average of three observations.

Of course no definite value can be obtained in respect to these differences as it is difficult to compare the different aquaria on a basis of identical factors, on which this difference depends. Summing up the above investigations it can, however, be concluded that under normal conditions in an aquarium the difference of pH between the inflow and outflow water ranges within a limit of 1.0 in pH.

## 5) DISCUSSION.

Among the chemical properties of sea water the oxygen and pH factors are of significance in the sense that, through the respiration and photosynthesis of marine organisms, they are closely interrelated with each other. Photosynthesis, having no relation to the aquarium problem, has been ignored in the present case. The changes of both factors are still of much importance as seen from the stand point of physiology. Why this is so, is due to the fact that the  $\text{CO}_2$  which affects the pH of water is evolved from oxygen consumption of fish, while the oxygen consumption is controlled by the  $\text{CO}_2$  (in this case pH), which decreases the oxygen-combining power of fish blood.

As just stated, the amount of  $\text{CO}_2$  evolved by the respiration of fishes can be calculated from the oxygen consumption if the respiratory quotient of aquarium animals is known. On the other hand the amount of  $\text{CO}_2$  can be found graphically from pH according to McCLENDON (1917). Based on these ideas a brief discussion will be made in respect to the gas condi-

tion of tank water. In the present experiment the water surface of the tanks was kept open instead of sealed as is usually done in the respiratory study of fishes. So that the result may be no more than showing the quantitative relations of the experiment in which the  $O_2$  and  $CO_2$  were allowed to invade or evade from the surface of water.

From the result of Exp. II (Table I) it will be noted that in the average of eleven tanks about 1.44 cc of oxygen was absorbed by fishes every minute. If, therefore, the R. Q. of fishes is assumed to be 0.8, about 1.15 cc of  $CO_2$  should be evolved in the water by the respiration of the fishes. According to McCLENDON (1917) 1.15 cc of  $CO_2$  per litre of water corresponds to about 0.07 in pH when the excess base is 24.5 cc. Consequently the pH which was 8.30 at first should decrease to 8.23 on account of the increase of  $CO_2$ , but instead of finding such a slight decrease we found a marked decrease, down to pH 7.82. When the  $CO_2$  is calculated from the decrease of pH (pH 0.48) it amounts to 7.43 cc as the pH 0.1 corresponds to 1.53 cc of  $CO_2$ . According to the calculation it follows that there is a difference of about 5.97 cc between the calculated and the observed amounts. If, on the other hand, the 7.4 cc of  $CO_2$  was actually secreted the R. Q. of fishes must be 5.2. This is unreasonable.

Oxygen entering from the surface of the water might account for at least part of the discrepancy. Because the oxygen infused into the water from surface, in addition to the oxygen in the inflow water, might also play a significant part in producing  $CO_2$ . Another large factor may possibly be the decrease of pH caused by the acid substance secreted by fishes. This is probable as the excreta of fishes may stagnate for a long while on account of the slow exchange of tank water, thus decreasing the alkali reserve of the sea water. In this regard BROWN (1929) also states that the "organic acid which are produced during metabolism neutralize some of the excess base, thereby reducing the buffering power of the water.

According to the data of my unpublished experiments of certain marine fishes made by using the closed circulatory system, the  $CO_2$  production measured by the pH method shows considerable error. This is caused by the inaccuracy of the colorimetric reading of pH which can not be greater than 0.05 in pH. pH 0.1 corresponds to about 1.53 cc of  $CO_2$ ; hence it is impossible to detect a difference smaller than 0.75 cc ( $CO_2$ ) when the pH method is used for the purpose of determination. From what has so far been stated it must be conceded that it is not at all easy to make a quantitative study of gas exchange by using the above mentioned method.

In respect to the attainable low pH of tank water ATKINS (1922) states

that when the water is in such condition some of its inmates would die. When the pH which had previously been 7.6, reached pH 7.3 symptoms of distress appeared among the fishes. In STOWELL's (1925) experiment none of the tanks showed pH below 7.70, but BROWN (1929) found the pH so low as 7.02 in a tank containing dogfishes. According to MC CLENDON (1917) the tension of  $\text{CO}_2$  becomes 6.2 mm when the pH of sea water drops to 7.02, provided the excess base i.e. alkaline reserve maintains its normal value.

The lowest pH observed in the present experiment was 7.6, in Tank No. 6, which was the most crowded of all. As a pH of 0.1 corresponds to 1.53 cc (per L) of  $\text{CO}_2$  the decrease of pH from 8.3 to 7.6 means an increase of 10.71 cc (per L) of  $\text{CO}_2$ . Though it was not clear whether this was due to the oxygen deficiency or to the low pH the Pogies in this tank showed an apparently laborious respiration. Comparing this pH to ATKINS's result, mentioned above, the symptoms of distress appeared at a much higher pH than in his experiments. This may have been due to the low oxygen content of the breathing water.

As will be seen from Table I a comparison of the oxygen consumption in Exp. I with that of Exp. II shows that the consumption in the latter case (1.33 cc per minute) was less than in the former case (1.73 cc per minute) by 0.4 cc per minute. This difference cannot be regarded as being due solely to the difference of oxygen tension in the breathing water in both experiments, inasmuch as the present experiment was not made in a closed circulation system. This difference may be due to the difference of the rate of oxygen invasion from the atmosphere into the tank water in the two experiments.

In closing the author expresses his cordial thanks to Prof. J. F. MC CLENDON for the valuable criticism.

#### SUMMARY.

- 1) The respiratory conditions of aquarium water were studied laying stress on the change of oxygen and pH changes of the circulatory water.
- 2) The rate of the decrease of oxygen content in water varies with the speed of the circulating water. Under the aforementioned conditions the mean decrease of oxygen per litre per minute is respectively 0.99 cc and 1.44 cc when the speed of water is 1.47 litres and 0.92 litres per minute.
- 3) The pH decrease of water also varies with the speed of circulating

water. Taking the mean value the rate of pH decrease per minute is respectively 0.34 and 0.48 when the speed of water is 1.47 litres and 0.92 litres per minute respectively.

4) The lowest oxygen content of tank water registered during the experiment was 2.096 cc per litre (Tank No. 6. Exp. II). The mean oxygen content of the outflow water varied from 3.84 to 3.31 cc when the speed of water flow varied from 1.74 to 0.92 litres.

5) The lowest pH of outflow water varying from 7.96 to 7.82 when the speed of water flow varied from 1.74 to 0.92 litres.

6) From the calculation due to pH change the  $\text{CO}_2$  evolved in tank water by fishes is 10.71 cc per litre per minute. When this is the case the fishes seem to show symptoms of distress as the oxygen supply is more or less decreased.

7) In the aquarium conditions under review the fishes can be kept normally by a water supply of 1-2 litres per minute.

8) During the aquarium season (May to November) the water temperature seasonally changes from about 9°C (early in May) to about 24°C (mid-August). The change of specific gravity of the water ranged between about 21.00 to about 25.00, during the same season.

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# THE CAUDINA OF ASAMUSHI, THE SO-CALLED CAUDINA CHILENSIS (JOHS. MÜLLER).

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(With Pls. V-VIII and 2 text-figs.)

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Of the many intricate problems concerning Molpadid-classification that of the specific limitation within the genus *Paracaudina* is one of the most interesting and for the present one of the most confused, a confusion which has led to a very deplorable wrong determination of the Japanese species so much used for studies in the anatomy, physiology, and biology of Molpadids.

In 1925 Dr. MORTENSEN showed (Echinoderms of New-Zealand and the Auckland-Campbell Islands IV. pp. 363-367)<sup>1)</sup> that the three species *chilensis* JOHS. MÜLLER, *coriacea* HUTTON, and *australis* SEMPER are well limited species, rather easily distinguishable, and he furthermore pointed out that the Japanese form *ransonetii* v. MARENZELLER could not be the same species as *chilensis* as maintained by H. L. CLARK in "The Apodous Holothurians"<sup>2)</sup>. In 1928 HÔZAWA published his beautiful study of the anatomy of *ransonetii* v. MARENZELLER<sup>3)</sup>, using the name *chilensis* JOHS. MÜLLER for the species, evidently without knowing MORTENSEN's paper. On Dr. MORTENSEN's calling his attention to the fact that the name he had used could not rightly be used for the Japanese form, Prof. HÔZAWA handed Dr. MORTENSEN's letter to Prof. OHSHIMA, who then in a pamphlet named "The *Caudina* of Asamushi"<sup>4)</sup> pointed out that neither the systematic characters used by MORTENSEN nor the way in which he used them were able to change the standpoint reached by Prof. CLARK and adopted by OHSHIMA, BENHAM, and JOSHUA & CREED.

During the studies of the large collections of Molpadids from the

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<sup>1)</sup> Papers from Dr. MORTENSEN's Pacific Expedition, XXIX, Vid. Med. Dansk Naturh. Forening, Bd. 79, 1925.

<sup>2)</sup> Smithsonian Contributions to Knowledge, Vol. XXXV, 1908.

<sup>3)</sup> Science Rep. of the Tôhoku Imperial University, Fourth Series, Biol., Vol. III, No 3, Fasc. 2, 1928.

<sup>4)</sup> Annot. Zool. Japonenses, Vol. 12, No. 1, 1929.

Danish "Ingolf"-Expedition, from Dr. MORTENSEN's collections, and from the German "Valdivia"-Expedition the present author has come to the conviction that MORTENSEN is right in using the calcareous deposits and the calcareous ring in the way he has done it in the named paper, and I wanted then to undertake an examination of the Japanese Molpadids and form my own opinion of the question whether the Japanese species is really identical with *chilensis*, as maintained by CLARK and OHSHIMA against the opinion of MORTENSEN. By the great kindness of Prof. HÔZAWA I received an exceedingly fine material of *ransonetii* from Japan and Prof. ARNDT, Berlin, gave me the opportunity of reexamining the type specimens of JOHS. MÜLLER's *Molpadia chilensis*. I beg here to express my sincerest thanks to these two gentlemen. Unfortunately some Australian specimens promised me more than a year ago, have not arrived, but as the problem mainly concerns the Japanese and the South-American forms I think it better not to await the problematic arrival of the Australian material and not to put off publishing the results of my studies any longer.

## I.

### Some Critical Remarks to our Present Knowledge of "Caudina" *chilensis* (JOHS. MÜLLER).

In "The *Caudina* of Asamushi" (Annot. Zool. Jap., 12, 1929) OHSHIMA first points out how important this "*Caudina*" is, being the only Molpadid which is rather easily collected and thus the only one which may be used regularly for scientific researches and experiments. And so it is, indeed, used; more than ten memoirs are published dealing with the physiology, biochemistry, development, anatomy, and ecology of this Holothurian. In all these memoirs the species is named *Caudina chilensis* (JOHS. MÜLLER), based as OHSHIMA states "upon my (his) identification of the specimens".

Thereupon the taxonomical difficulties of the species *chilensis* are shortly mentioned and then, where the reader expects that OHSHIMA is going to settle these problems he shortly states that he "has no intention to settle the problem now in this short note, but some critique of MORTENSEN's paper may be of no little use". He states further that this critique is not based upon own studies, but are "chiefly based on HÔZAWA's recent work ("Calcareous deposits of *Caudina chilensis*" 1928) which is a result of careful examination on a great number of specimens, and thus well to be trusted". HÔZAWA's work is indeed exceedingly fine, and having examined the specimens sent by Prof. HÔZAWA himself the present author

is able to state that it is anatomically seen quite correct. In spite of this it is quite evident that HÔZAWA, having his attention devoted particularly to the "Changes occurring with advancing age in the calcareous deposits" of this species, and apparently not having any thorough knowledge of the classification of the group, has not clearly seen what is classificatorily characteristic of the species examined. He describes the different shape of the calcareous ring within the four developmental stages, but he does not point out which characteristics show that it is in all the four stages a ring of that special species and not of any other, and he gives numerous figures of calcareous deposits but without pointing out clearly which shape is the really characteristic one and may be found in all the different stages. In Pl. XIV, fig. 4 HÔZAWA figures how low and broad the interradials may be in small specimens and in Pl. XVII, fig. 22 how narrow and high they may be in old specimens, and the comparing of these two figures led OHSHIMA to the supposition that it is wrong to use the calcareous ring as a systematic character. However, the examination of the specimens at hand does not show so great a variation, the smallest specimens having a narrower and the largest specimens (being larger than that figured by HÔZAWA) having a wider interradial than shown in HÔZAWA's figure. And as to the deposits HÔZAWA usually does not figure the normal ones, only the much modified, though normal ones may be found also in these large specimens.

In criticising MORTENSEN, OHSHIMA states that "one must note that it is by no means safe to decide the species distinction by simply examining a few illustrations of calcareous deposits;—MORTENSEN gives four figures of calcareous deposits for *coriacea* and three for each of *chilensis* and *australis*, among them one for each species is in side-view". To this it must be said that one has little reason to suppose that MORTENSEN has decided from his few figures and not from his examination of the numerous deposits in the preparations. The comparison of the specimens of the different species at hand has convinced me that MORTENSEN is right in using the shape of the deposits as specific characters, though they may be rather alike and often difficult to separate for an untrained eye.

As OHSHIMA remarks (p. 42) that the size of MORTENSEN's specimens is not given, I may here state that the size of MORTENSEN's specimens is: *australis*—6.5 cm., *coriacea*—10.5 cm., and *chilensis*—16.0 cm. OHSHIMA suggests that "judging from the types of those deposits (i.e. those figured by MORTENSEN) we may infer from HÔZAWA's result that the *coriacea*-type represents the youngest, the *chilensis*-type the medium sized, while the



*australis*-type represents the oldest stage; if we suppose that the three species are really but one and the same species". This suggestion accordingly does not hold very well.

As to the calcareous ring OHSHIMA states: "MORTENSEN tries to take the shape of the calcareous ring *as one of the characteristics to distinguish Holothurian species*. So marked a structure, and so diversely formed, this organ is apt to lead one to regard it as of too much importance in taxonomy. My impression is that *the form and structure of the calcareous ring do show characteristics of each species*, but are not in accordance with natural affinity of groups, being rather variable, due to adaptation to the modes of life". Contrary to OHSHIMA I must however maintain that in any case within the Molpadids the shape of the calcareous ring gives, in spite of changes due to age, the best base for separating genera and families. In this connection it does not help to take examples from the other families of Holothurians e.g. from the *Cucumaria* and *Phyllophorus*, as done by OHSHIMA. Further, though I am not able to state much about the value of the ring within these groups, I suppose that a closer study of the anatomy of the species belonging to these groups will result in a division of them corresponding to differences in the shape of the calcareous ring.

On page 430 OHSHIMA states that: "MORTENSEN seems to attach importance to the shape of the anterior margin of the radial segments of the calcareous ring. It is a general rule among the Holothurians that possesses 15 tentacles, irrespective of whether they be *Molpadiidae* or *Cucumariidae*, that the radial segment of *the calcareous ring has its anterior margin divided by a projection into two unequal indentations*.... The position of the median projection, whether it shifts to either extreme or remains not far from the middle, and the relative size of the two indentations thus formed, are not so constant as MORTENSEN appears to consider. One can see that it is quite a variable feature even in HÔZAWA's illustrations (figs. 4, 9, 15, 22)".

To this I am obliged to say that OHSHIMA appears to have not quite understood the building up of the radials within the Molpadids. They do not have their anterior margin "divided by a projection into two unequal indentations", but they have two projections, normally separated by a medial indentation, and the one of the projections, the muscular projection (i.e. that to which the longitudinal muscles are fastened) has usually a passage for the nerve, either a perforation (e.g. in the genus *Molpadia*) or a notch (e.g. most species of *Paracaudina*). Such a passage may be lacking (e.g. in *coriacea* as stated by MORTENSEN; he has not overlooked

any notch as OHSHIMA supposes).

The building up of the calcareous ring in the Molpadids is the most easily understood when one goes out from the ring in a Synaptid with 15 tentacles (e.g. *Pendecaplectana*). In such a specimen there are 5 radials and 10 interradials placed in such a way that a tentacle may be inserted between each two pieces. When in such a ring each of the radials coalesces with one interradial, we get a ring with five interradials and with five radials, each of which latter consists of two parts of different origin, which is indicated by the presence of two anterior projections, or perhaps better by the presence of insertions for one whole and two half tentacles, (cf. Pl. VIII, fig. 1) i.e. a normal Molpadid-ring, and when instead of the hole in the radials there is only a notch for the passage of the nerve and the radial canal, we have a normal ring of a *Paracaudina* e.g. of *P. ransonetii* (v. MARENZELLER). That such an alteration may really take place is seen within the Chiridotids and the Myriotrochids. All the Chiridotids with 12 tentacles have 12 pieces in the calcareous ring, and some species have some or all the radials notched and not perforated (e.g. *Chiridota pisanii* LUDWIG). In the Myriotrochids with 12 tentacles there are only 10 pieces, but the two dorsal radials are obviously composed of two pieces, one of radial and one of interradial origin. From the diagrammatical figures (Pl. VI, fig. 14-18) it is easily seen how the shape of the calcareous ring is to be understood, and how the tentacles are placed between the anterior tips of the pieces, indicated by the arrows in fig. 18.

As to the so-called *Cuvierian organs* found by JOHS. MÜLLER and refound by MORTENSEN, the present author may only state that they exist, without being able to say what they are. OHSHIMA states that "it is not very clear from his (MORTENSEN's) statement whether he did find such in MÜLLER's type specimens", but as he uses these organs as characters usable for classification and denies their presence in the two other species it seems evident that he has seen them in the type of *chilensis*.

From what is stated above it would seem evident that the reasons given by MORTENSEN are at any rate so much more weighty than those of OHSHIMA, especially as MORTENSEN has decided from the examination of specimens, and a well understood examination too, whereas OHSHIMA has only decided from the rather few figures given by MORTENSEN and HÔZAWA; which he himself states is "by no means safe".

As it will appear from the following description of the characters usable in the classification of this genus, MORTENSEN is quite right in his supposition that *chilensis* MÜLLER, *australis* SEMPER, *coriacea* HUTTON, and *ran-*

*sonetii* v. MARENZELLER are separate species, and thus the problem of "*Caudina chilensis* (JOHS. MÜLLER)" is—I think—definitely settled, and that in the way pointed out by MORTENSEN in 1925.

## II.

### Description and Comparison of the Systematical Characters of "*Caudina*" *chilensis* and Allied Species.

When dealing with the classification of Molpadids, one of the greatest problems is, which characters are usable for separating genera and species. The different apprehensions hereof are nearly as many as are the students of the group. Naturally the only safe way is to take all characters, macro- and micro-anatomical as well as eidonomical, into consideration, but even in doing so the great variation of the characters and the fact that often only few specimens are available<sup>1)</sup> afford great difficulties.

As the appearance of the specimens is usually dependent on the way of preservation and the degree of contraction, and the greater part of the anatomy is often quite spoiled by the strong contraction, the main character used is the shape of the calcareous deposits; beside this, several authors have used the shape of the calcareous ring. As a matter of fact the combination of these two characters may in nearly all cases serve to distinguish the species with a rather high degree of certainty, and when further a few other characters are taken into consideration, the determination of a specimen may be nearly quite certain.

In the case of "*Caudina*" *chilensis* OHSHIMA, of course, admits that the shape of the calcareous deposits serve as a good systematical character, but according to HÔZAWA's work he does not suppose that the differences pointed out by MORTENSEN in 1925 are sufficiently clear to allow a distinction between different forms (species?); the use of the calcareous ring for specific distinction he will not admit. A closer examination and especially a careful comparison of the deposits and of the calcareous ring, the retractor muscles and the genital papilla, definitely show that MORTENSEN is right in his distinction between the species. For showing this clearly a description and a discussion of the named characters are necessary.

a) *Calcareous Deposits*. In "The calcareous deposits of *Caudina chilensis*"<sup>1)</sup> HÔZAWA states that the fully developed spicules are "rings which enclose a cross on the one face and a square on the other, the cross

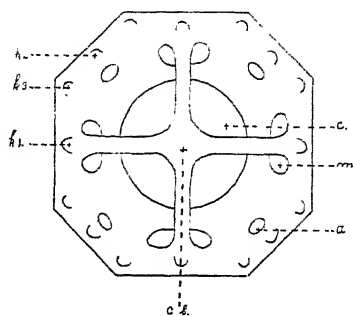
<sup>1)</sup>This title is an abbreviation of: On the Changes occurring with Advancing Age in the Calcareous Deposits of *Caudina chilensis* (Y. MÜLLER), proposed by HÔZAWA himself.

and square being scarcely separated from each other". This description of the deposits is seemingly quite correct, but a closer examination with a high magnification ( $\times 1800$ ), as well as a comparison with other Holothurians shows that it leads to a wrong apprehension of the deposit, which further has occasioned OHSHIMA's difficulties in distinguishing between the different forms.

The normal fully developed deposit or "button" (Text-fig. 1) consists of a little octagonal plate, the basal plate, with five perforations, a large usually circular central-hole (*c.*) and four oblong marginal holes (*m.*). Over this plate and, as HÔZAWA states, always on the exterior surface is a low "spire" in the shape of a cross-bridge (*c.b.*), placed with the four stems at the outer margin of the marginal holes. This arrangement of the cross-bridge quite agrees with the way in which the bridge is placed over the side holes in the anchor-plates of the Synaptids (*e.g.* of *Synaptula*). (As the cross-bridge is developed directly of the primary cross or better *is* the primary cross, it is not at all homologous with the bridge in the Synaptid plate, only its arrangement according to the marginal holes is the same as that of the Synaptid bridge to the side holes of the anchor-plates). When compared with the deposits of other Holothurians the "buttons" of *Paracaudina* thus quite resemble the tables of some aspidochirote forms as these are drawn by EKMAN in 1925 (*Systematisch-phylogenetische Studien über Elaspipoden und Aspidochiroten*) p. 436, Fig. C. b; only the crown is totally lacking.

In normal plates we thus do not find twelve holes as stated by HÔZAWA, but only five, the large number being only found when in abnormal plates the cross bridge is more closely united with the basal plate, being then placed in the same plane and not over it. Besides the mentioned five perforations some few additional holes (*a.*) may be found, even in quite normally developed plates, but the appearance of such holes is usually combined with anomalies in the shape of the basal plate.

The margin of the basal plate is more or less regularly undulating



Text-fig. 1. Diagrammatical figure of a calcareous deposit from the body-wall of a *Paracaudina* (*ransonettii*)

*a.*—additional hole.

*c.*—central hole.

*c.b.*—cross-bridge.

*k*<sub>1</sub>–*k*<sub>3</sub>.—knobs of 1st to third order.

*m.*—marginal hole.

and the exterior surface of it is often supplied with knobs ( $k$ ). These knobs are rather regularly placed and may according to their place and occurrence be named knobs of first, second or third order ( $k_1$ ,  $k_2$ ,  $k_3$ ), and in this way that the knobs opposite the stems of the cross bridge are of the first order, those opposite the additional holes of the second and those between the marginal and the additional holes are of the third order.

Such normal deposits are figured by HÔZAWA in his different plates, but besides those he has shown a large number of more or less deviating ones, without clearly pointing out which is the real typical shape. In Pl. V, fig. 1-2 I have drawn two quite typical deposits from a little specimen belonging to HÔZAWA's "first stage", and in the figures 3-18 are shown deposits from gradually larger specimens. It is obvious that the deposits are different in the different specimens, and a joint correlation with the size of the specimens may be found, but this is not quite so striking as figured by HÔZAWA. In the largest specimens in which according to HÔZAWA the deposits are all typically diverging from those of the smaller specimens, deposits may easily be found which, except for the surrounding yellow substance, quite agree with those found in quite small ones (cf. fig. 1-2 and fig. 17-18). That the larger part of the deposits in old specimens are abnormal in shape and furthermore are surrounded by the interesting clear yellow stuff, is, seen from a classificatory point of view, of no great importance as the typical and for the species quite characteristic shape of deposits may be found in all specimens, the smallest as well as the largest.

When we now compare the deposits of the other species of the genus with what is found in the Japanese one (*ransonetii* (v. MARENZELLER)), it is evident that they are distinctly different. The deposits of *coriacea* (HUTTON) are very regular (incomplete and thus irregular ones are naturally found, but I only speak of the typical and for the species characteristic ones) and exceedingly uniform (Pl. VI, figs. 8-13). In the one specimen (that from New Brighton) the deposits (Pl. VI, figs. 10-13) are all thick and "fat" and without knobs, but in the specimens from Tiri-Tiri the deposits (Pl. VI, figs. 8-9) are more slender and with large "fat" knobs which distinctly differ from the knobs found in *ransonetii* and *chilensis* (Pl. VI, figs. 1-4). It is true that some deposits from *ransonetii* may be rather like those in *coriacea* (cf. HÔZAWA Pl. XIV, fig. 2 c and Pl. XVI, fig. 11 c), but when a larger number of deposits are compared the differences are quite clear. As it also appears from the figures in Pl. V, the deposits of the specimens from Tiri-Tiri rather definitely differ from those of the specimens from New Brighton, and probably the specimens really

belong to two different forms of *Paracaudina*. The scanty material at hand does however not allow a clearing up of this problem, and as to the problem dealt with in this paper it is really sufficient to show that *coriacea* (this species being composed of two varieties or not) is at any rate clearly different from *ransonetii*, *australis*, and *chilensis*.

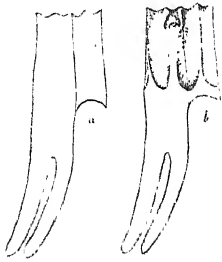
The deposits of *chilensis* (JOHS. MÜLLER) (Pl. VI, figs. 1-4) are as those of *coriacea* rather thick, but they have not the "fat" appearance, and when they have knobs these are more conspicuous.

The deposits of *australis* (SEMPER) (Pl. VI, figs. 5-7) differ distinctly from those of *coriacea* and *chilensis* as also shown by MORTENSEN in 1925, as the cross bridge is not developed, and the deposits themselves are rather irregular and supplied with a varying number of holes and knobs. They are to some degree like some of the abnormal deposits found in *ransonetii* (cf. HÔZAWA Pl. XIV, fig. 2 b) but also here the typical appearance of the deposits is very different, and moreover deposits with well developed cross-bridge do not seem to occur in *australis*.

As it appears from this description a careful comparison of deposits from the four species concerned allows a clear distinction between them, and the few deposits in one species which may strikingly resemble some of the deposits in another species may only be regarded as indicators of the common genus to which they all belong. Such cases of resemblance are indeed found within most genera of apodous Holothurians.

b) *Calcareous Ring*. As shown by HÔZAWA the appearance of the calcareous ring may alter during the life of the specimen, but in spite of this the real, characteristic shape is strikingly constant. It appears that HÔZAWA has cleaned the calcareous pieces too much before drawing, in that the insertions of the tentacular muscles and the tentacle ampullae are removed. In spite of this it is evident also from HÔZAWA's figures that the radials have two anterior projections one of which is distinctly notched for the passage of the nerves and radial canals. This process, to which the retractor is fastened, we may designate as the *muscular process*, the other accordingly the *non-muscular process*. The impressions for the insertions of the tentacle muscles are rather large, but not so deep as those of *chilensis* and *coriacea*. The insertions for the retractor muscles are rather deep and in the largest specimens there may be found a faint furrow from the insertion of the retractor muscle to the anterior notch (Pl. VII, fig. 3). In *chilensis* (Pl. VII, fig. 5) the insertion for the retractor muscle is large and deep, which nicely conforms with the long and well developed retractors; a real notch is absent, as the furrow from the

muscular insertion to the top of the projection is broad and deep. All the muscular impressions in the surface of the ring are very deep and



Text-fig. 2. Radial and inter-radial piece of the calcareous ring in *Paracaudina chilensis*.

- a. The figure given by MORTENSEN in "The Echinoderms of New Zealand etc." p. 366 c, showing only the outlines of the pieces.
- b. Quite the same figure with addition of the characteristic sculpture.

more conspicuous than those in the ring of *ransonetii*. On comparing my figure (Pl. VII, fig. 5) with that given in fig. 47, c of Dr. MORTENSEN's paper it would seem that the latter must be incorrect. This is, however, not the case; the differences are mainly due to the fact that in the said figure no attention has been paid to the sculpture of the calcareous ring, only to the differences in the anterior outline found in the various species. I am reproducing here (Text-fig. 2) the said figure from Dr. MORTENSEN's paper and together with it the same figure with addition of the sculptural impressions. These two figures clearly show that the figure from MORTENSEN's paper (Fig. 47, c) in reality corresponds very well with mine (Pl. VII, fig. 5).

The calcareous ring of *australis* (Pl. VII, figs. 8-9) differs distinctly from that of the two named species (as also from that of *coriacea*) in having very faint impressions for the tentacular muscles, and in having a very sharp and narrow, deep notch in the muscular processes. Furthermore it differs in a curious way from the other rings in the top of the interradial and the radial non-muscular processes being free of the fascias for the tentacle muscles.

In *coriacea* Pl. VII, figs. 6-7 the radials have no notch in their muscular process, as also stated by MORTENSEN in 1925. OHSHIMA states "that MORTENSEN found in *coriacea* only two lateral projections leaving a wide concavity between, leaves room for a suspicion that he overlooked the presence on top of one of the projections of a minute notch to receive the radial canal". I have very carefully examined MORTENSEN's preparation, and must declare that he is right in his statement, and that the muscular process prepared free by him is nicely rounded on the top, leaving not the faintest trace of a notch. However, in order to be quite certain in this question I prepared the other radials free and found that one of the others has, not a faint notch, but a faintly flattened part on the tip of the muscular process (Pl. VII, fig. 7). Thus it is seen that the calcareous ring

in *coriacea* really differs from that in the other species in having no notch, but in some cases a faint trace of the passage of the radial nerve may be present. The sculpture in the surface of the ring is very deep and distinct.

As it appears from what is stated above the calcareous ring of the four species concerned differs in a way which is clearly beyond the degree of specific variation and thus affords the strongest character for separating the species, and, as it nicely corresponds with the differences in the shape of the calcareous deposits, clearly shows that both characters are to be trusted within this genus.

c) *Retractor Muscles*. As to retractor muscles of Molpadids, CLARK in 1908 states (p. 144) that "the formation of such retractors appears to be very uncommon, if not an altogether exceptional event, possibly only occurring in certain individuals, perhaps very old ones, and the presence or absence of such retractors cannot be considered as having any value in taxonomy". CLARK is thus far right in what he states, as the presence of retractors is altogether exceptional within the Molpadids. They were first found by JOHS. MÜLLER in *chilensis*, and by him, LUDWIG and others used as one of the characteristics of the genus *Molpadia* (= *Paracaudina*, as this genus was understood for many years). In reality I have never found retractors in specimens belonging to other genera than JOHS. MÜLLER's *Molpadia* (= *Paracaudina*), but all the four species here dealt with have distinct retractors, though developed in a different degree. The retractors are in *ransonetii*, in small as well as in large (old) specimens only faint, being developed from the longitudinal muscles which have their outer edges turned up and coalescing so as to form like a small funnel (Pl. VIII, fig. 2). In *coriacea* (Pl. VIII, fig. 3) they are very distinct though short, (in a 6 cm long specimen not more than 0.5 cm) and as in *ransonetii* united with the longitudinal muscles by a rather solid web, which, however, does not contain muscles. The two retractors from each pair of longitudinal muscles join before they reach the calcareous ring (Pl. VIII, fig. 3). In *australis* the retractors of a 6.5 cm long specimen are 1.5 cm long. They are united with the longitudinal muscle by a very thin membrane which contains some few fine, but distinct muscles. The two retractors do not join, but are attached each to a limited place on the calcareous ring (Pl. VIII, fig. 6). In *chilensis* the retractors (Pl. VIII, figs. 4-5) are in a 16 cm. long specimen 1.2 cm. long. They are by a thin web, which does not contain muscles, attached to the exterior margin of the longitudinal muscles (Pl. VIII, fig. 5). The two retractors unite as



they reach the calcareous ring.

d) *Genital Papilla*. It is obvious that marked differences in the genitalia have to be regarded as some of the strongest indicators for systematic difference, but it must be admitted that only through careful studies of the state of the genital papilla in living, mature specimens from the different seasons of the year, can we form a definite judgment of the classificatory value of this organ. Thus one is not allowed to use differences found in the genitalia exteriora of a few poorly preserved specimens as characters of much value, though they ought to be mentioned. In all the specimens of *ransonetii* there is a long genital papilla, which now and then is somewhat contracted, but always quite distinct. This papilla is also mentioned as a character of the species by MITSUKURI in his Studies of Actinopodous Holothurioidea. In the specimens of *chilensis* there is but a short and not much contracted papilla, and in *coriacea* a papilla is lacking. In *australis* the preservation of the specimens does not allow any statement concerning the presence or absence of a genital papilla.

e) *Cuvierian Organs*. So-called Cuvierian organs are found by JOHS. MÜLLER in the type of *chilensis*, and they are refound by both MORTENSEN and the present author. What they really are I cannot tell, but they are rather distinct. There is nothing to support the suggestion by OHSHIMA (p. 44) that they might be gregarines. Such organs do not appear to occur in the other species. However, further investigations on fresh material of *chilensis* is needed before we shall be able to use these organs as a systematic character.

### III.

#### Discussion of the Systematic Position of the four Forms *chilensis* JOHS. MÜLLER, *coriacea* HUTTON, *ransonetii* MARENZELLER, and *australis* SEMPER.

From the above description it definitely appears that we have four different forms of Molpadids, previously described under the names *chilensis*, *coriacea*, *ransonetii*, and *australis* and in modern time confused under the common name *chilensis*. Neither CLARK nor OHSHIMA are willing to see any differences between these four forms and only Dr. DEICHMANN (cfr. OHSHIMA 1929, p. 45) suggests that there is a difference, at least between the Japanese and the South-American forms, and therefore suggests that the Japanese form reasonably may be called *chilensis* var. *ransonetii*.

Naturally it may be a matter of personal taste, whether the four forms

should be regarded as distinct species or only as distinct varieties of one large species. Distinct they are at any rate and to my mind there can be no question but that they should be regarded as distinct species. Besides the morphological differences, zoogeographical reasons speak decidedly\* against regarding them all as varieties only of *chilensis*. The distribution from Japan to Australia-New Zealand could offer no serious objection. But the fact that no form, which could be referred to *chilensis* is known to occur along the West coast of North-America and Central America would be quite unintelligible, were the Chilean form really identical with the Japanese-Australian-New Zealand forms. No reasonable explanation can be given of such a "zoogeographical paradox".

As to the question where among the other Molpadid species these four are to be placed, there can be no doubt that they form their own genus within the family *Caudinidae*. This is no new conception of mine, as several authors have distinguished between this genus, naming it *Molpadia* (as done by JOHS. MÜLLER) and STIMPSON's genus *Caudina*, and it was first CLARK who in 1908 united them under the name *Caudina*. As the name *Molpadia* obviously was wrongly used by JOHS. MÜLLER, LUDWIG and others for this genus, I in 1931 proposed the name *Paracaudina* for it (first using the name *Pseudocaudina* which, however, was preoccupied).

The genus *Paracaudina* differs distinctly from *Caudina* in the shape of the calcareous deposits, in the presence of retractor muscles and perhaps also in the arrangement of the mesenteries.

The species may be distinguished thus:

1. Calcareous deposits in medium sized specimens very varying, normally without a well developed cross-bridge. Radials of calcareous ring with very sharp and deep incision, and the anterior processes of the ring with free tips.....*australis*.  
In spite of great variation, calcareous deposits with a cross-bridge never quite lacking. Incisions of radial pieces not so sharp and deep, and anterior processes of calcareous ring not with their tips free. ....2
2. Muscular process of radials without notch, and without furrow between muscular insertion and tip of projection. Deposits very regular, usually "fat", with or without thick, rounded projections. Genital papilla absent.....*coriacea*  
Muscular process of radials notched, or with a deep furrow from the insertion of the retractors to the tip of the projection. Deposits not "fat" and rounded. Genital papilla present. ....3

3. Retractor muscles well developed, long. .... *chilensis*  
 Retractor muscles funnel-shaped, very short. .... *ransonetii*

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## EXPLANATION OF LETTERS.

- a.l.m.* Anteriormost part of longitudinal muscle.  
*c.m.* Coalesced part of retractor muscle.  
*f.p.r.* Bifurcate posterior prolongation of radial pieces.  
*f.t.m.* Fascias of tentacle muscles.  
*i.p.* Inserting part of retractor muscle.  
*i.r.* Interradial piece of calcareous ring.  
*i.r.m.* Insertion mark of retractor muscle.  
*i.r.p.* Interradial process of anterior margin of calcareous ring.  
*i.t.m.* Insertion mark of tentacle muscle.  
*l.m.* Longitudinal muscle.  
*l.v.i.a.* Left ventral interambulacrum.  
*m.d.i.a.* Mid-dorsal interambulacrum.  
*m.p.* Muscular process.  
*n.m.p.* Non-muscular process.  
*n.v.* Notch in anterior margin of muscular process.  
*r.* Radial piece of calcareous ring.  
*r.m.* Retractor muscle.

- t.a.* Tentacle ampulla  
*t.m.* Tentacle muscle.  
*u.w.* Web uniting retractor muscle with longitudinal muscle.

## Pl. V.

Deposits of *Paracaudina ransonetii* (JOHS. MÜLLER).

Figs. 1-2 from a specimen measuring 1.5 cm. (1st stage)

„ 3-4	„ „ „ „	4.0 „ (2d „ )
„ 5-6	„ „ „ „	7.5 „ (3d „ )
„ 7-10	„ „ „ „	9.5 „ (3d „ )
„ 11-13	„ „ „ „	22 „ (4th „ )
„ 14-18	„ „ „ „	27 „ (4th „ )

The deposits Fig. 12 and Figs. 14-18 are surrounded by the yellow stuff mentioned by HÔZAWA.

## Pl. VI.

Figs. 1-4 Deposits of *Paracaudina chilensis*; type specimen.

„ 5-7	„ „ „	<i>australis</i> .
„ 8-9	„ „ „	<i>coriacea</i> , specimen from Tiri Tiri.
„ 10-13	„ „ „	„ „ New Brighton.
„ 14-17	Diagrammatical figures of calcareous rings in	

Fig. 14 *Molpadia*

„ 15 *Paracaudina*

„ 16 *Pendecaplectana*

„ 17 *Myriotrochus*.

The punctured part of the radials in figs. 14-15, and 17 as well as in fig. 18 is that which may be regarded as an interradial coalesced with a true radial. The punctured interradial in fig. 16 is that which in a Synaptid may be regarded as corresponding with the non-muscular part (the punctured) of a Molpadid (and a Myriotrochid).

Fig. 18 Diagrammatical figure showing the arrangement of the pieces in the calcareous ring of a Molpadid; the shading is the same as in Figs. 14-17. As the tentacles are always placed *between* the anterior tips of pieces (See the arrows in fig.), it is seen from the figure that there are four tentacles in the middorsal interambulacrum, two in the left ventral and three in each of the others.

## Pl. VII.

Radial and interradial pieces of calcareous rings in

Figs. 1-3 *Paracaudina ransonetii*

Fig. 1 from a 1.5 cm. long specimen

„ 2	„ „	9.5 „ „ „
„ 3	„ „	27.0 „ „ „

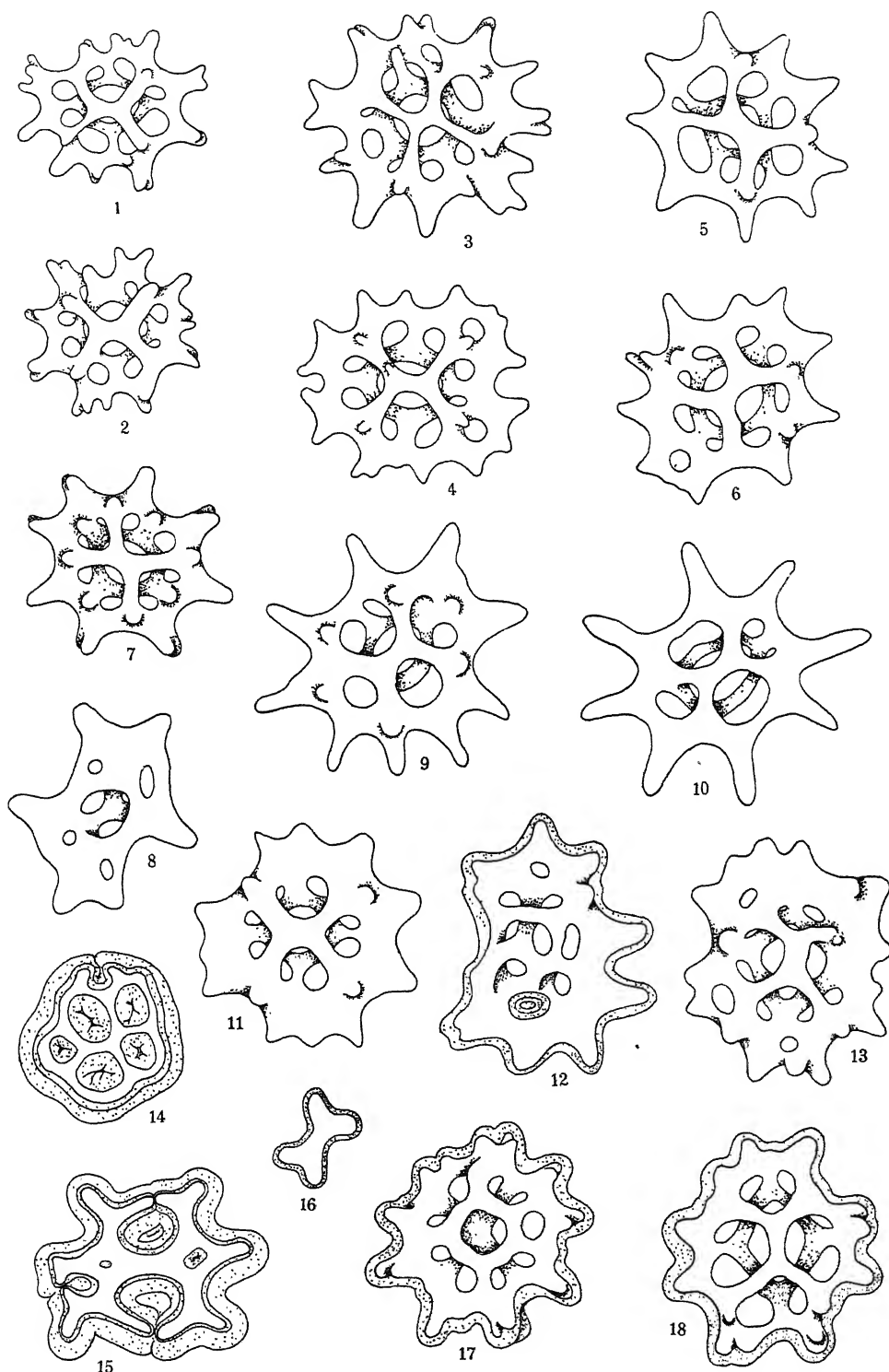
Figs. 3-5 *Paracaudina chilensis*

„ 6-7 „ „ *coriacea*

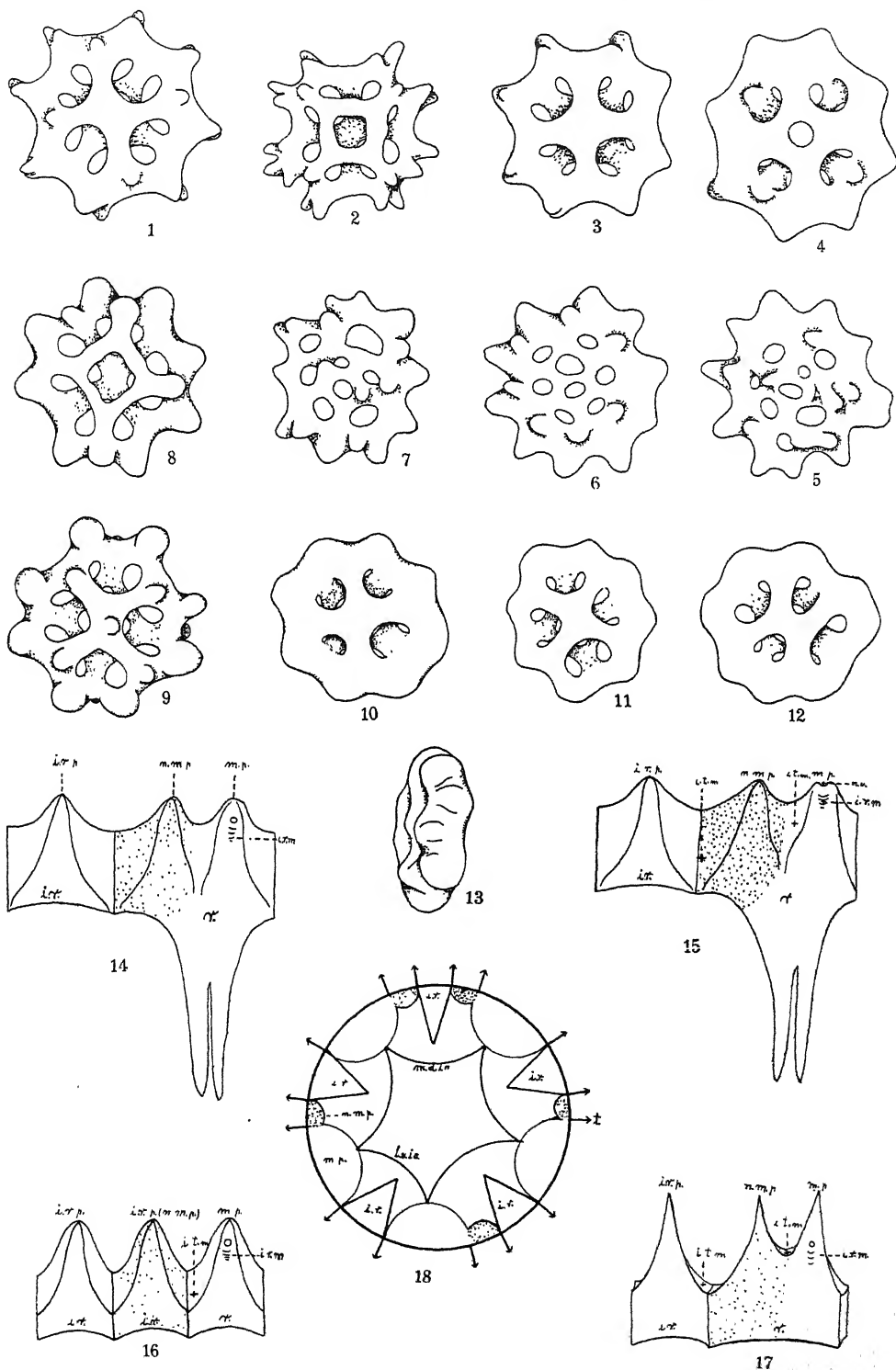
„ 8-9 „ „ *australis*

## PL. VIII.

- Fig. 1 *Paracaudina ransonetii*, a radial and an interrarial piece of the calcareous ring showing the arrangement of the tentacles.
- „ 2 *Paracaudina ransonetii*, anterior part of longitudinal muscle with retractor
- „ 3 *Paracaudina coriacea*, „ „ „ „ „ „ „
- „ 4 „ *chilensis* „ „ „ „ „ „ „
- „ 5 „ „ Diagrammatical section of half part showing arrangement of longitudinal muscle and retractor.
- „ 6 „ *australis* anterior part of longitudinal muscle with retractor.

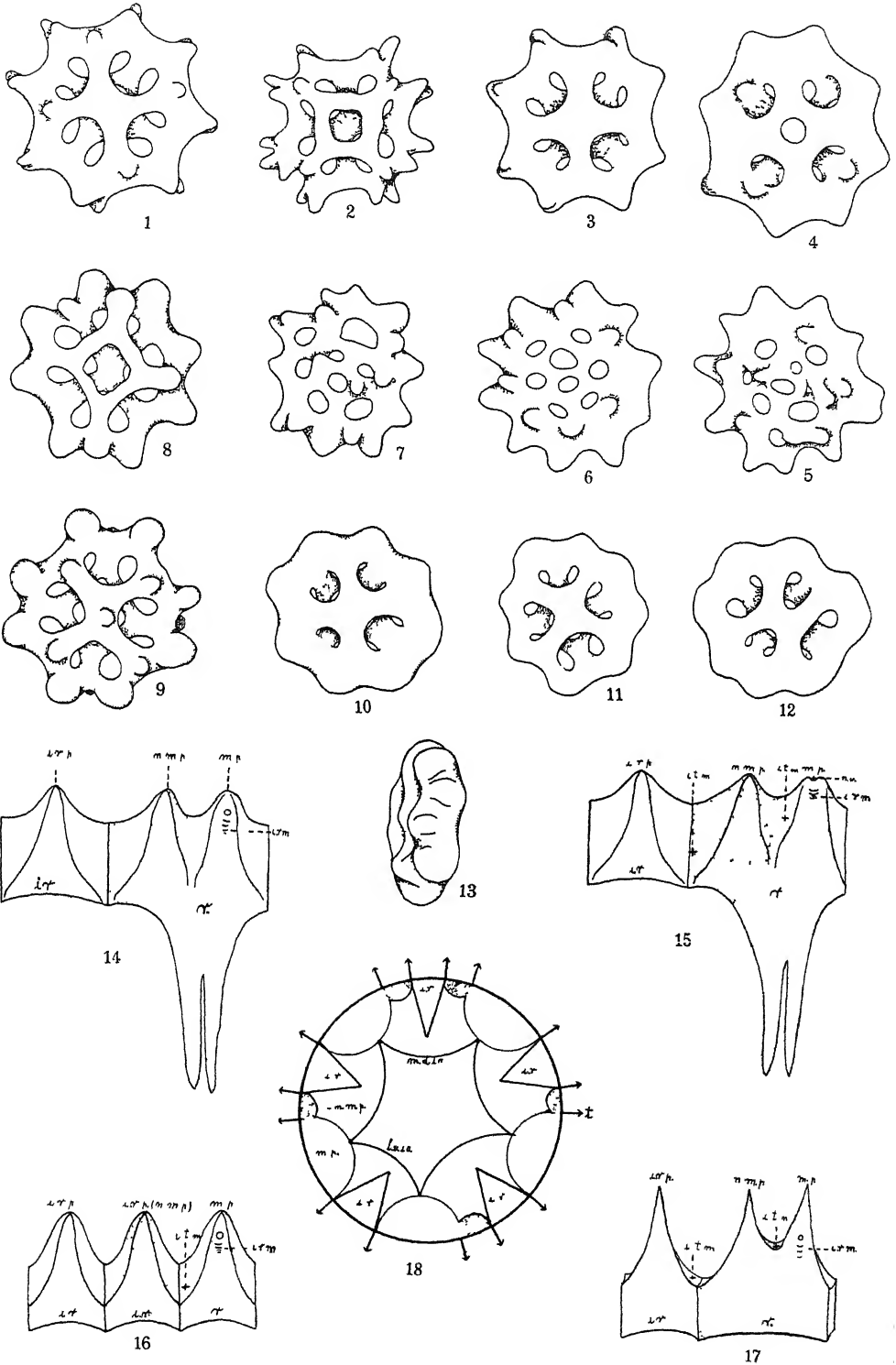




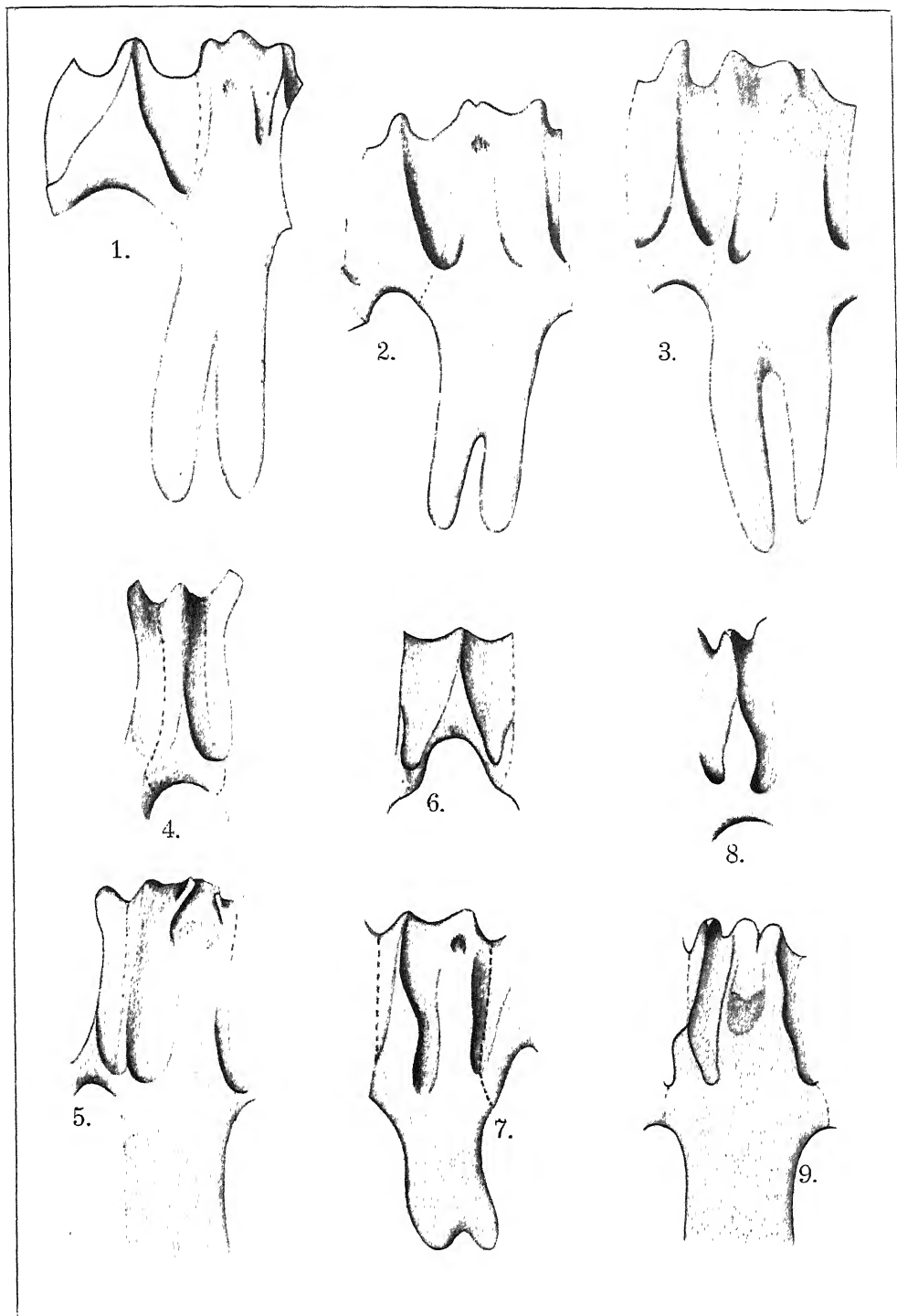




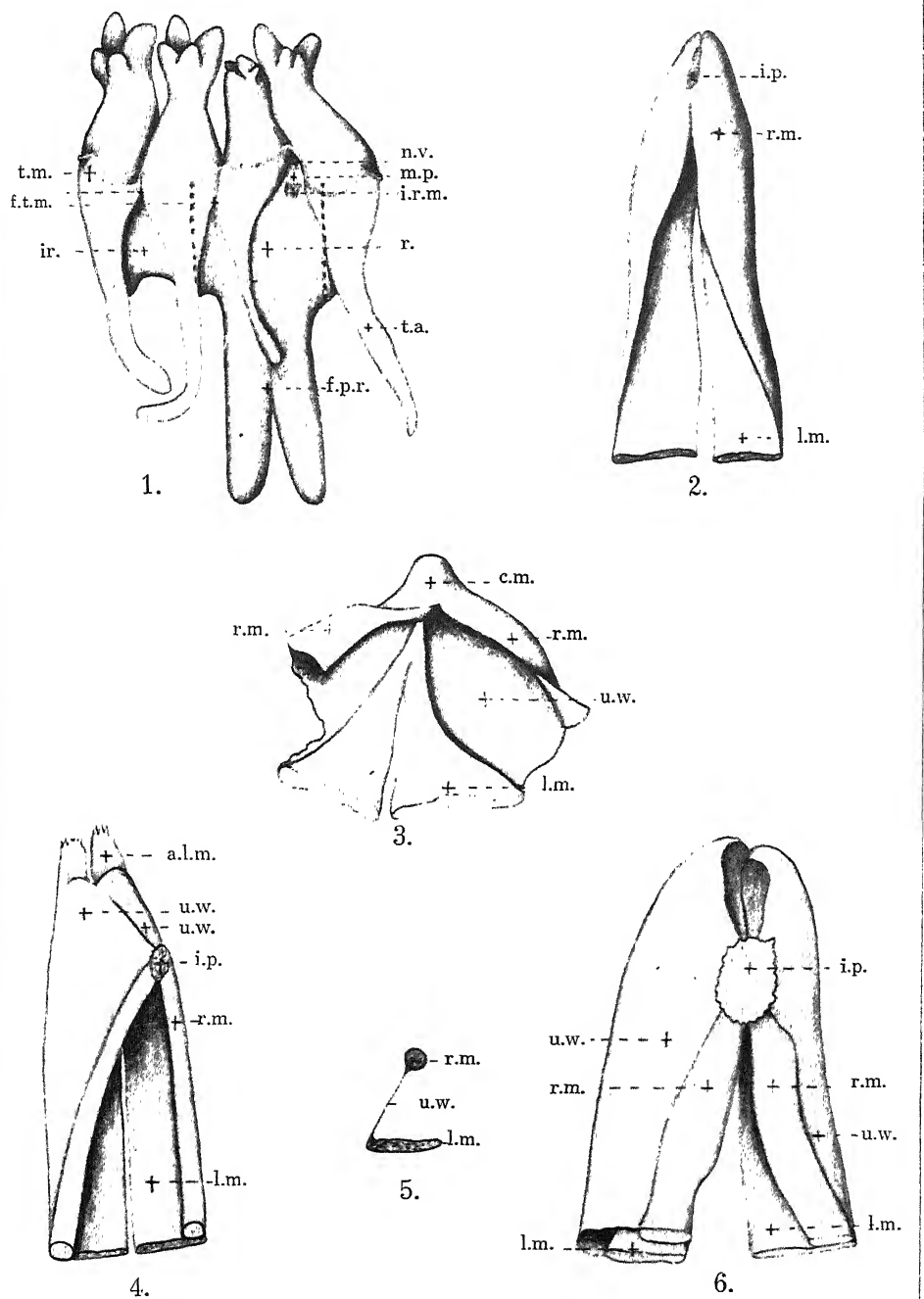














# ON THE ASYMMETRICAL GROWTH IN THE SHELLS OF *SANGUINOLARIA OLIVACEA* JAY.

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(With two figures)

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The specimens of the marine bivalve, *Sanguinolaria olivacea*, here dealt with were collected from the sand between the tidal lines at Matsukawa-Ura near Haragama, Fukushima Prefecture, May, 1930. They were kindly identified by Mr. SHICHIHEI NOMURA.

The shells of the species under discussion are asymmetrically formed, the left valve being taller and deeper than the right. When viewed from the anterior side, most of the individual shells show a convex line of meeting of the valves towards the left side of the animal body (Fig. 1, R), owing to this asymmetrical growth. The object of the present study was to determine how the asymmetrical growth of the valves was attained.

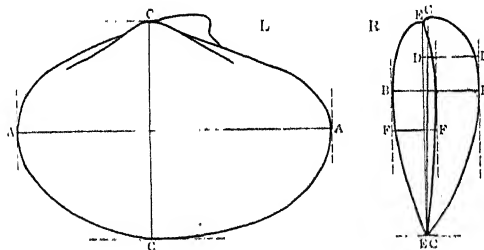


Fig. 1. Schematized representation of *Sanguinolaria olivacea*, to illustrate the points at which the measurements were taken. Much magnified. L—left-side view, R—anterior view.

AA—antero-posterior length (A)

BB—dextro-sinistral width (B)

CC—dorso-ventral height of the left valve (C)

DD—depth of the left valve (D)

EE—dorso-ventral height of the right valve (E)

FF—depth of the right valve (F)

The longest antero-posterior length (AA) and the widest dextro-sinistral width (BB, perpendicular to AA) of the shells, the respective dorso-



ventral height of the left (CC) and right (EE) valves, and in determining the respective depth of the left (DD) and right (FF) valves 470 specimens were measured by Mrs. Y. HORIMI. The height of the left valve shows, exactly, that of the individual shell, and the length of the shell is, exactly, that of both valves on the left and right of the shell.

The results of the measurements are given in Table 1.

TABLE 1.

Length (A) in mm.	Number of shells of same length measured	Measurements averaged in mm.				
		Width (B)	Left valve		Right valve	
			Height (C)	Depth (D)	Height (E)	Depth (F)
14.0	1	4.2	10.7	2.4	10.7	2.2
16.9	1	4.8	12.6	2.8	12.5	2.3
18.0	2	5.0	13.3	2.9	13.2	2.5
18.7	1	5.4	14.5	2.8	14.3	2.7
19.5	1	6.2	15.4	3.3	15.3	3.0
20.3	2	5.9	15.2	3.3	15.0	2.8
20.5	2	5.9	15.3	3.3	15.1	2.9
21.0	4	6.2	15.9	3.5	15.9	3.1
21.2	1	6.4	15.5	3.7	15.3	3.2
21.3	1	6.2	15.6	3.5	15.5	2.8
21.4	2	6.4	15.7	3.9	15.6	3.1
21.6	2	6.5	16.6	3.7	16.5	3.0
21.7	1	6.3	16.0	3.6	15.7	3.0
21.8	1	6.7	16.4	4.0	16.2	3.0
21.9	3	6.5	16.6	3.9	16.4	3.1
22.0	2	6.6	16.5	3.6	16.3	3.2
22.2	1	7.0	16.7	4.0	16.5	3.2
22.3	1	7.0	16.6	4.0	16.2	3.2
22.4	3	6.9	16.8	4.0	16.6	3.2
22.5	2	7.0	17.0	4.0	16.9	3.4
22.6	3	6.7	17.1	4.0	16.9	3.1
22.7	3	6.9	17.5	3.8	17.4	3.4
22.8	1	7.1	16.7	4.1	16.5	3.1
23.0	5	6.9	17.3	4.0	17.0	3.3
23.2	2	6.7	17.5	4.0	17.4	3.0
23.3	4	6.9	17.4	4.0	17.3	3.3
23.4	3	6.9	17.3	3.9	17.1	3.3
23.5	2	7.2	17.8	4.2	17.5	3.4
23.6	5	7.0	17.8	4.0	17.6	3.3
23.7	3	7.4	17.8	4.5	17.6	3.6
23.9	4	7.3	17.7	4.3	17.4	3.6
24.0	6	7.1	17.6	4.2	17.4	3.3
24.2	1	7.0	18.2	4.0	18.0	3.2
24.3	5	7.2	18.1	4.2	17.9	3.3
24.4	1	7.4	19.0	4.4	18.9	3.4
24.5	4	7.2	18.5	4.3	18.2	3.4
24.6	3	7.2	18.2	4.3	17.9	3.2
24.7	6	7.2	18.3	4.3	18.1	3.4
24.8	8	7.4	18.6	4.4	18.4	3.4
24.9	5	7.3	18.9	4.4	18.6	3.6
25.0	12	7.5	18.7	4.5	18.4	3.4
25.1	1	7.3	18.8	4.1	18.6	3.5

Length (A) in mm.	Number of shells of same length measured	Measurements averaged in mm.				
		Width (B)	Left valve		Right valve	
			Height (C)	Depth (D)	Height (E)	Depth (F)
25.2	3	7.5	18.9	4.5	18.6	3.4
25.3	6	7.6	18.6	4.5	18.3	3.5
25.4	6	7.7	19.0	4.6	18.7	3.6
25.5	9	7.6	19.0	4.4	18.7	3.5
25.6	4	7.7	19.5	4.5	19.2	3.6
25.7	4	7.6	19.6	4.3	19.5	3.6
25.8	1	8.0	19.5	4.8	19.4	3.9
25.9	4	7.8	19.3	4.6	19.0	3.6
26.0	12	7.9	19.4	4.7	19.1	3.7
26.1	2	7.7	19.3	4.6	19.1	3.5
26.2	4	7.8	19.6	4.6	19.3	3.5
26.3	7	7.9	20.1	4.8	19.7	3.6
26.4	5	7.8	19.8	4.6	19.5	3.7
26.5	8	8.0	19.8	4.8	19.5	3.6
26.7	5	8.0	19.6	5.0	19.3	3.5
26.8	9	8.0	19.8	4.8	19.5	3.7
26.9	9	8.0	20.1	4.6	19.8	3.8
27.0	10	8.1	20.2	4.8	19.9	3.7
27.1	2	8.2	20.5	5.1	20.1	3.7
27.2	7	8.0	20.5	4.7	20.3	3.8
27.3	5	7.9	20.2	4.9	20.0	3.6
27.4	8	8.2	20.2	4.7	19.9	3.9
27.5	7	8.6	20.6	5.1	20.3	4.0
27.6	5	8.0	20.4	4.8	20.1	3.7
27.7	4	8.5	20.8	5.0	20.5	3.9
27.8	10	8.3	21.0	4.9	20.7	3.9
27.9	1	8.4	21.1	5.2	20.7	3.8
28.0	12	8.6	21.1	5.1	20.8	3.9
28.1	1	8.3	21.1	5.0	20.7	3.8
28.2	7	8.8	21.2	5.2	21.0	4.1
28.3	9	8.6	21.4	5.1	21.1	3.9
28.4	6	8.9	21.4	5.2	21.1	4.1
28.5	5	8.7	21.3	5.0	20.9	4.1
28.6	4	8.4	20.9	5.1	20.7	3.7
28.7	5	8.6	21.3	5.1	21.0	3.9
28.8	6	8.9	21.5	5.2	21.2	4.1
28.9	6	8.7	21.5	5.3	21.1	4.0
29.0	11	8.7	21.8	5.2	21.5	4.0
29.1	4	9.0	22.0	5.5	21.7	4.2
29.2	2	9.0	21.9	5.3	21.7	4.3
29.3	7	9.2	21.8	5.5	21.5	4.2
29.4	6	8.8	21.7	5.1	21.4	4.0
29.5	4	9.0	21.3	5.4	21.0	4.0
29.6	10	9.1	22.3	5.5	21.9	4.1
29.7	1	9.1	22.4	5.5	22.2	4.3
29.8	4	9.4	22.2	5.7	21.9	4.3
29.9	2	9.5	22.6	5.6	22.3	4.5
30.0	11	9.0	22.1	5.4	21.8	4.0
30.2	1	9.6	22.4	5.8	22.0	4.1
30.3	4	9.3	22.7	5.7	22.2	4.2
30.4	5	9.0	22.8	5.3	22.5	4.2
30.5	3	9.2	23.0	5.5	22.3	4.2
30.6	3	9.0	22.5	5.6	22.1	4.0
30.7	2	9.4	23.0	5.7	22.7	4.3
30.8	3	9.4	23.1	5.7	22.8	4.2
30.9	4	9.6	23.2	5.6	22.8	4.5

Length (A) in mm.	Number of shells of same length measured	Measurements averaged in mm.				
		Width (B)	Left valve		Right valve	
			Height (C)	Depth (D)	Height (E)	Depth (F)
31.0	6	9.7	23.5	5.9	23.0	4.4
31.1	1	9.2	22.8	5.6	22.7	4.4
31.2	2	9.6	23.3	5.8	23.0	4.4
31.4	3	9.6	22.8	5.6	22.6	4.5
31.5	1	11.0	24.4	6.8	23.9	4.6
31.7	3	10.2	23.6	5.8	23.1	4.8
31.8	1	9.8	24.3	6.0	23.9	4.4
31.9	1	10.0	24.4	5.9	24.3	4.4
32.0	4	9.7	23.9	5.9	23.6	4.4
32.1	1	10.0	23.1	5.8	23.0	4.8
32.2	2	9.7	24.1	5.9	23.7	4.4
32.3	3	10.0	23.8	5.6	23.5	4.6
32.4	1	9.8	24.8	6.0	24.5	4.2
32.5	1	10.0	23.7	6.1	23.2	4.5
32.6	1	9.8	24.4	6.0	24.0	4.0
32.7	2	10.3	24.4	6.2	24.1	4.8
32.9	1	10.0	24.0	6.0	23.8	4.6
33.0	1	10.3	25.0	6.0	24.5	4.9
33.1	1	10.5	25.1	6.3	24.8	4.6
33.3	1	10.3	23.9	6.3	23.5	4.6
33.6	2	10.4	25.4	6.2	24.7	4.7
33.8	1	10.0	24.6	5.9	24.1	4.4
34.2	1	10.0	25.6	6.0	25.1	4.4
34.4	1	10.9	25.4	6.7	25.2	4.7
35.0	1	11.2	25.5	6.8	25.2	5.0
36.7	1	11.7	27.0	7.0	26.5	5.0

## RESULTS OF THE CALCULATIONS.

The length, because of its being the longest measurement, was taken as the abscissa in making the calculations which were worked out by Assistant J. OIZUMI, Mrs. HORIMI, Miss K. IKEUCHI, and by myself. The following equations were obtained<sup>1)</sup>:

$$1) B=0.218 A^{1.10}$$

$$2) C=0.748 A^{1.00}$$

$$3) D=0.109 A^{1.15}$$

$$4) E=0.788 A^{0.98}$$

$$5) F=0.149 A^{0.98}$$

The equations are expressed in Fig. 2.

<sup>1)</sup>NOMURA, E. 1926. An application of  $a=kb^x$  in expressing the growth relation in the freshwater bivalve, *Sphaerium heterodon* PILS. Sci. Rep. Tôhoku Imp. Univ., 4th ser., Vol. 2, Pp. 57-62.

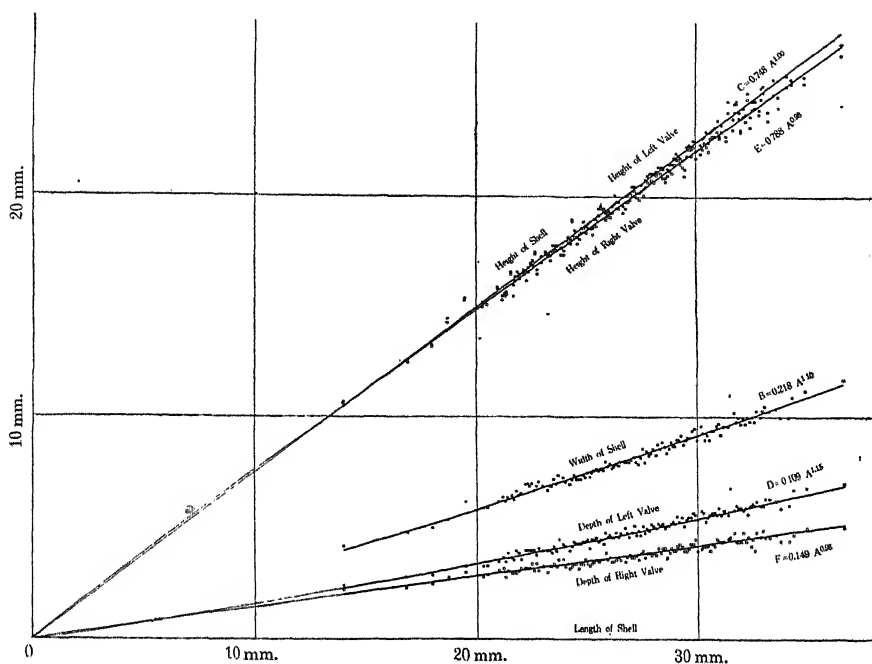


Fig. 2. Plottings from Table 1, and expressions of Equations 1)-5).

From these, the following equations are derived:

From 1),	6) $A=3.999 B^{0.91}$	
From 2),	7) $A=1.337 C^{1.00}$	
From 3),	8) $A=6.877 D^{0.87}$	
From 4),	9) $A=1.275 E^{1.02}$	
From 5),	10) $A=6.972 F^{1.02}$	
From 6) and 7),	11) $B=0.301 C^{1.10}$	12) $C=2.991 B^{0.91}$
From 6) and 8),	13) $B=1.814 D^{0.96}$	14) $D=0.536 B^{1.04}$
From 6) and 9),	15) $B=0.283 E^{1.12}$	16) $E=3.067 B^{0.89}$
From 6) and 10),	17) $B=1.842 F^{1.12}$	18) $F=0.583 B^{0.89}$
From 7) and 8),	19) $C=5.144 D^{0.87}$	20) $D=0.158 C^{1.15}$
From 7) and 9),	21) $C=0.954 E^{1.02}$	22) $E=1.048 C^{0.98}$
From 7) and 10),	23) $C=5.215 F^{1.02}$	24) $F=0.198 C^{0.98}$
From 8) and 9),	25) $D=0.144 E^{1.17}$	26) $E=5.218 D^{0.85}$
From 8) and 10),	27) $D=1.016 F^{1.17}$	28) $F=0.987 D^{0.85}$
From 9) and 10),	29) $E=5.289 F^{1.00}$	30) $F=0.189 E^{1.00}$

## DEDUCTIONS FROM THE EQUATIONS.

The length of the shell grows at a more rapid rate in comparison with that of the height (9) and of the depth (10) of the right valve, but at a slower rate in comparison with the width (6) of the shell and of the depth (8) of the left valve, while it grows at the same rate as the height (7) of the shell.

The width of the shell grows at a more rapid rate in comparison with that of the length (1) and height (11) of the shell, and of the height (15) and depth (17) of the right valve, but at a slower rate as compared with the depth (13) of the left valve.

The height of the shell grows at a more rapid rate in comparison with the height (21) and depth (23) of the right valve, but at a slower rate compared with the width (12) of the shell and with the depth (19) of the left valve, while it grows at the same rate as the length (2) of the shell.

The depth of the left valve grows at a more rapid rate in comparison with the length (3), width (14) and height (20) of the shell, and the height (25) and depth (27) of the right valve.

The height of the right valve grows at a slower rate in comparison with the length (4), width (16) and height (22) of the shell, and the depth (26) of the left valve, while it grows at the same rate as the depth (29) of the right valve.

The depth of the right valve grows at a slower rate in comparison with that of the length (5), width (18) and height (24) of the shell, and the depth (28) of the left valve, while it grows at the same rate as the height (30) of the right valve.

## REMARKS.

The left and right valve are equal in length, and this length is exactly that of the shell. From this relation, we may infer accurately that the height and depth of the right valve grow at a slower rate in comparison with that of the height and depth of the left valve, and we obtain the ratios :

$$\frac{C}{E} = 0.949 A^{0.02} \quad \text{from 2) and 4), and}$$

$$\frac{D}{F} = 0.732 A^{0.17} \quad \text{from 3) and 5).}$$

However Equations 2) and 4) intersect each other at the point where  $A=13.5$  mm. and  $C=E=10.1$  mm., and Equations 3) and 5) at the point where  $A=6.3$  mm. and  $D=F=0.9$  mm. These relations suggest that, in

a shell shorter than 13.5 mm. in length, the left valve is lower than the right in height, and that, in a shell shorter than 6.3 mm., the left valve is shallower than the right in depth. If these expressions were true, the asymmetrical growth of the valve ought to be reversed according to data found in the case of both younger and older specimens. Actually, however, in the younger specimens again collected by Mr. OIZUMI from the same locality at Matsukawa-Ura, on January 13, 1933, the left and right valves are symmetrically grown. Thus, for specimens shorter than 13.5 mm. in length, I wish to express the proportions by the equation

$$C=E=0.748 A^{1.00},$$

and, for specimens shorter than 6.3 mm. by the equation

$$D=F=0.143 A^{1.00}.$$

In relation to the method of measuring the depths of the valves, it is evident that the sum of these depths is not equal to the width of the shell. But in specimens shorter than 6.3 mm. the sum shows directly the width of the shell. Therefore the equation

$$D+F=B=0.286 A^{1.00}$$

may be applied to the younger specimens.

#### CONCLUSIONS.

1. In specimens of *Sanguinolaria olivacea* JAY shorter than 6.3 mm. in the length (antero-posterior) of the shell, the left and right valves are symmetrically formed.

2. In specimens longer than 6.3 mm., the asymmetrical growth of the valves begins: that is, the depth of the left valve becomes deeper than that of the right, even though the height of each valve may still grow equally at the same rate as that of the length until the length reaches 13.5 mm.

3. In specimens longer than 13.5 mm., the left valve alone continues to grow in height at the same rate as that of the length, and the height of the right valve becomes lower than that of the left.



PHYSIOLOGICAL STUDIES ON *DROSERA*.  
IV. ON THE FUNCTION OF MICRO-ORGANISMS IN THE  
DIGESTION OF INSECT BODIES BY INSECTIVOROUS PLANTS.\*

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I. INTRODUCTION.

In regard to the mechanisms of the disintegration and digestion of insect bodies by insectivorous plants, we find in the scientific reports two main divergent views. According to one, the decomposition takes place owing to the action of enzymes secreted by the plants themselves, while the other tends to lay stress upon the rôle of micro-organisms.

As early as in 1875, MORREN reported that the disintegration of insect bodies and proteins deposited on the leaves of *Pinguicula* and *Drosera* might be caused by the action of the bacteria and fungi which were found by the microscopic observation made several days afterwards. A similar record was made by BATALIN in 1876 for *Drosera*. Thereafter MORREN abandoned his previous idea, because in the case of *Drosera binata* even though the protein had dissolved no micro-organisms were perceptible.

The rôle of micro-organism in the disintegration of insect bodies has also been emphasized by TISCHUTKIN and DUBOIS. TISCHUTKIN (1889, '92) after twenty-four hours, observed a large number of bacteria in the fluid which was secreted by the stimulation of deposited sterilized protein in the leaves of *Pinguicula vulgaris*, *Drosera rotundifolia*, *Dr. longifolia* and *Dionaea muscipula*, and found that these micro-organisms were capable of attacking peptones. Further, he noted that, in the fluid secreted in the unopened pitchers of *Nepenthes Mastersi*, neither bacteria nor proteolytic action were perceptible while in the one of the mature pitchers the contrary was the case. For this reason, he concluded that the secreted fluid of insectivorous plants may merely be a nutrient medium for micro-organisms, and that the decomposition of proteins to reptonones may be due to the action of bacteria. Following TISCHUTKIN, DUBOIS (1890) made researches on

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\*) Contributions from the Mt. Hakkôda Botanical Laboratory. No. 18.



the physiology of some species of *Nepenthes*, especially on the process of the protein decomposition. Using a similar method to that of TISCHUTKIN he found no pepsin in the secreted fluid of the *Nepenthes*-pitchers, so that the disintegration of insect bodies should be attributed to the organisms invading them from outside, and not to the substance secreted by the plants.

An eclectic theory combining both views has been suggested by LABBÉ and STUTZER. LABBÉ (1904), indeed, isolated some organisms, e. g. *Aspergillus glaucus*, *Penicillium glaucum*, *Mucor mucedo*, *Cladosporium herbarum*, *Micrococcus cinnabarius*, *Bac. aureus*, *Bact. termo* and *Bact. lincola*, from the leaves of *Drosera rotundifolia*. He assumed that the fungi, which were the most dominant among the organisms isolated, may oxidise glucose in the secreted fluid to form an acid, while the latter may succeed in activating the trypsin-like enzymes of *Drosera*. More recently, STUTZER (1926) noted that the process of nutrition through the disintegration of insect bodies may as much depend upon the propagation of bacteria which belong to the *Bac. coli* group as upon the proteolytic enzymes of the plants themselves, and the residual bacteria may be subject to the action of the *coli*-group.

Recently, however, it has been clearly settled that the insectivorous plants secrete a proteolytic enzyme, so that the collaboration of micro-organisms may seem to be superfluous. Accordingly, in this work the present writer has taken up this question anew to throw some light on the whole process of the digestion of insect bodies by insectivorous plants.

In his treatment of the subject, the present writer has directed his researches towards the physiological characteristics of some species of micro-organisms, isolated from the leaves of *Drosera rotundifolia*, and from the inside of the pitchers of *Nepenthes mirabilis*, and to their relation to the decomposition of nitrogenous compounds. Indeed, it may also be mentioned here that in these plants the tests for organisms which were able to attack the nitrogenous compounds, were always positive. Therefore, it seems very probable that these organisms may take part in the complete decomposition of insect bodies in the insectivorous plants, for the proteolytic enzymes found in the leaves of these plants, so far as these studies reach, can only decompose the proteins up to the peptones.

## II. EXPERIMENTS.

### A. Isolation of Micro-organisms in *Drosera rotundifolia*.

Five leaves of *Drosera* which had been cultivated in a green-house and

had neither caught any insects nor held any other foreign matter, were gathered, washed in sterilized water, and the liquid was then diluted with a given quantity of water, and made to inoculate in an ordinary gelatine plate. The total number of colonies of organisms, after four days inoculation was calculated to be 500 to 1,400 individuals on a leaf. Repeated experiments showed that in most cases the number of fungi exceeded that of the bacteria, though in a few cases this result was reversed. The organisms found in every case are given below. Both their morphological and physiological characteristics were studied according to the technique of the Society of American Bacteriologists, some important points regarding which may be stated as follows:

1. *Bac. albolactis*. \*

Motile with flagella. Spores central. GRAM positive. Gelatine stab: rapid crateriform to infundibuliform liquefaction. Agar slant: white, wrinkled. Litmus milk: acid, slow coagulation. Starch-agar: intensely hydrolyzed in 3 days. Indol and hydrogen-sulphide: negative. Ammonia reaction, (DUNHAM's solution used), positive. Hydrolyzes glucose, lactose, sucrose, maltose, and fructose, giving acid and gas.

2. *Bac. glaveolens*.

Non-motile. GRAM positive. Gelatine stab: slow crateriform liquefaction. Agar slant: white, viscid, wrinkled. Litmus milk: weak acid, slow coagulation and peptonization. Reduces nitrates. Indol and hydrogen-sulphide reactions: negative. Ammonia reaction positive. Hydrolyzes glucose, sucrose, and maltose giving acid, but no gas production. Produced neither acid nor gas from lactose.

These bacteria have already been found in the soil. In addition to the above mentioned bacteria, *Mucor mucedo*, *M. racemosus*, *Phizopus nigricans*, *Penicillium glaucum*, *Aspergillus glaucus*, *Actinomyces* and yeast were also isolated. The classification of fungi is commonly based on the morphological characteristics at present, so that descriptions of them are not given here, though various observations must have been carried out for their identification.

B. Isolation of Micro-organisms in *Nepenthes mirabilis*.

For purpose of comparison with those from *Drosera* the isolation of organisms from the pitchers of *Nepenthes* was also carried out. For two to four days before opening the outsides of the pitchers were sterilized with mercuric chloride solution, one c.c. of the pitcher-fluid was drawn off with the sterilized injector (a glass needle was substituted for the metallic), and, without dilution, was made to inoculate in a gelatine plate. No colony of organisms was found after four days incubation. Repeated experiments always gave the same results. Therefore, it is reasonable to expect no organisms in the unopened pitchers. This fact is in accordance

with the reports of TISCHUTKIN and DUBOIS. But egg-albumin and edestin were hydrolyzed by the secreted fluid of the unopened pitchers, with the addition of hydrochloric or acetic acids. So that the proteolytic enzyme in the fluid of the unopened pitchers may be expected to exist in such a form as the pepsinogen from which the pepsin is formed.\* After the opening of the pitchers, insects or foreign matters has invaded them, and the number of micro-organisms rapidly increased with the lapse of time. After they had been open for seven days the total numbers of micro-organisms in one c.c. of the liquid contained by them was calculated to be from 3,800,000 to 37,600,000.

3. *Bact. gastricum*.

GRAM positive. Gelatine stab: rapid stratiform liquefaction. Agar slant: yellowish-white, filamentous, moist. Litmus milk: strong acid, rapid coagulation and peptonization. Reduces nitrates. Indol and hydrogen sulphide reactions negative. Ammonia reaction positive. Hydrolyzes glucose, sucrose, lactose, fructose, giving acid and gas.

4. *Bact. coli-anindolicum*.

GRAM negative: Gelatine stab: dark white, very slow crateriform liquefaction. Agar slant: white, viscid, filamentous. Litmus milk: acid, coagulated after 5-7 days. Reduces nitrates. Indol and hydrogen-sulphide reactions negative. Ammonia reaction positive. Produces gas and acid on glucose, sucrose, lactose, maltose and fructose.

5. *Bact. diffusum*.

GRAM negative. Gelatine stab: dark yellow, slow crateriform liquefaction. Agar slant: yellowish white, glistening. Litmus milk: weak acid, no coagulation. Reduces nitrates. Indol and hydrogen sulphide reactions negative. Ammonia reaction positive. Hydrolyzes starch, but produces neither gas nor acid from glucose, sucrose, lactose, maltose and fructose.

Among the bacteria isolated from *Nepenthes*, No. 3 and No. 4 have formerly been found in the intestinal canals of animals, and No. 5 in the soil and in water. In addition to the bacteria above-mentioned, fungi such as *Mucor mucedo*, *M. racemosus*, *Rhizopus nigricans*, *Aspergillus glaucus* and *Penicillium glaucum*, were also isolated from the pitchers of this plant as in the case of *Drosera*.

### C. The Action of Micro-organisms on Proteins, Peptides, and Amino Acids.

When proteins are decomposed by the action of micro-organisms, the first products are thought to be amino acids, which may be further deaminized to cause the formation of ammonia. In determining the rate of decomposition of proteins and amino acids, the amino- and ammonia nitrogen values are first calculated for use as the index. The researches in this direction gave the result that the organisms which decompose

\* The details in reference to this point will be given in the next reports on the physiology of *Nepenthes*.

nitrogenous compounds may be found in the *Drosera*-leaves as well as in the *Nepenthes*-pitchers.

a. *The Culture Media and Methods.*

The standard medium used in these experiments was of the following composition :

Water	1,000 c.c.
NaCl	5 g.
Na <sub>2</sub> SO <sub>4</sub>	2 g.
KH <sub>2</sub> PO <sub>4</sub>	1 g.
CaCl <sub>2</sub>	1 g.

This medium with the addition of some proteins was used by BAINBRIDGE (1911), and by BERMAN and RETTGER (1916) to culture bacteria. In my experiments, as the sole source of nitrogen and carbon casein, glycyglycine, glycocoll, or alanine was added to the above medium in the final concentration of one per cent, while in WITTE's peptone a concentration of two per cent was used. The pH of the secreted fluid of *Drosera* or *Nepenthes* is found to be about 3 to 6.\*<sup>1</sup> In this relation the acidity of the culture medium was kept at pH 5 to 6, while in the cases of peptone and of glycocoll pH 3,3 was also used as the medium.

Though in the case of BERMAN and RETTGER (1916), the bacteria were reported to grow only in the medium with an admixture of beef-extract, in my case, both bacteria and fungi grew without this addition. Contrary to the usual method in the culture of fungi, sugar was not added to the medium for the following reasons: 1) According to KENDALL (1915), BERMAN (1918) and others, the presence of the available sugar in the medium inhibits the decomposition of protein with the postponement of the formation of the proteolytic enzyme until the sugar is consumed. 2) The presence of sugar may cause some difficulty in the investigation of the decomposition products of amino acids.

Each 25 c.c. of the medium was poured into a mes-ERLENMEYER flask which had been plugged with cotton, and sterilized in autoclave, and it was then inoculated with various organisms after they had been cultivated on slant agar for 24 hours. The media not inoculated were used as controls. These flasks were left in the thermostat at 27-30°. In order to avoid the escape of volatile by-products—such as ammonia—without being previously sterilized by heating, it was added, 15 days after, with sterilized water to cover its loss by evaporation during the incubation, and

\*<sup>1</sup>) The details will be given in another report.

shaken and filtered. The content of amino nitrogen in the filtrates was determined by the VAN SLYKE's micro-method (ABDERHALDEN, Handb. der Biol. Arbeitsmeth., Abt. 1, Teil 7, S. 57). The ammonia nitrogen was determined by the distillation *in vacuo*, led to the flasks with given quantities of N/70  $\text{H}_2\text{SO}_4$ , and titrated with N/70 NaOH (ibid, S. 57); moreover, for the amino acids used, PERNAS and WAGNER's distillation apparatus (PREGL: Die quantitative organische Mikroanalyse, 1930) was applied. In both cases, each 2 c.c. of samples was used. The hydrogen-ion concentration was determined with the potentiometer by using the hydrogen electrode. The presence of ammonia in the sample causes an error in the determination of amino nitrogen by VAN SLYKE's method. According to PARSON and STURGES (1926), the amino nitrogen values obtained without the removal of ammonia are too high by 18 to 50 per cent of the total ammonia nitrogen present, the amount per cent depending upon the temperature and the quantity of the sample used. I, therefore, tried to remove the ammonia contained by heating the sample on the water-bath at  $40^\circ$  for 90 minutes, while passing air free from ammonia through the liquid. Though the formation of ammonia is taken as the index of the decomposition of proteins, and of their degradation products such as peptides and amino acids, some organisms may be able to attack the ammonia formed; and on the other hand, keeping the culture for some time in an aerobic condition at a certain temperature in a flask plugged with cotton may cause the loss of the ammonia formed. Further in the determination of amino nitrogen, the consumption of some parts of the amino acid as well as of the ammonia by organisms should be taken into consideration. In other words, the measurable values are only those of substances which accumulate in the medium, which is not utilized by the micro-organisms. Thus, some fluctuation in the results are unavoidable. The amino- and ammonia nitrogen values shown in the following tables are those calculated for 100 c.c. of culture medium.

b. *Experiments in the Medium pH 5-6.*

1. Casein.

3 g. of HAMMARSTEN's casein were dissolved in 10 c.c. of N/10 NaOH, the quantity being increased by the addition of the standard culture medium to 300 c.c. This medium was found to be of the required acidity. The results are given in Table I.

Table I shows that the majority of organisms, especially *Bac. albolactis*, *Penicillium*, and *Rhizopus*, attack the casein markedly; that is, a gain in

TABLE I.  
The decomposition of 1% casein.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	Gain in the sum of amino- and ammonia-N.	pH
		mg.	mg.	mg.	
1	Control	6.7	1.8	—	5.80
2	<i>Bac. albolactis</i>	49.4	68.5	109.4	8.35
3	<i>Bac. graveolens</i>	41.0	30.6	63.1	7.48
4	<i>Bact. gastricum</i>	25.6	48.4	65.5	8.40
5	<i>Bact. coli-anindolicum</i>	6.8	2.0	0.3	5.81
6	<i>Bact. diffusum</i>	6.7	1.8	—	5.80
7	<i>Asper. glaucus</i>	17.0	45.1	56.3	8.25
8	<i>Penic. glaucum</i>	18.6	66.8	76.9	8.80
9	<i>Mucor mucedo</i>	26.7	5.5	23.7	6.18
10	<i>M. racemosus</i>	65.8	12.9	70.2	6.94
11	<i>Rhizopus nigricans</i>	34.1	49.3	75.9	8.43

the sum of amino and ammonia nitrogen and marked changes in the hydrogen-ion concentration to the basic side are noticed, while in the case of *Bact. coli-anindolicum* and *Bact. diffusum* no change is found in the medium, the protein not being broken up. The degree of change in hydrogen-ion concentration on the whole, goes parallel with the amount of ammonia formed. While the final acidity of *Bac. albolactis* might be expected to be more basic than that of *Penicillium*, the contrary was found to be the case. Therefore, it is impossible to measure the degree of the decomposition of the proteins only by the determination of the hydrogen-ion concentration of the medium. In the cases of *Bac. albolactis*, *Bact. gastricum*, *Penic. glaucum* etc. the amount of measurable amino nitrogen is smaller than that of ammonia nitrogen, while the contrary is the case with *Mucor mucedo*, *M. racemosus* etc. This fact shows the difference in the relative non-liability to decomposition of amino acid, formed in the hydrolysis of casein, up to ammonia. The greater amount of amino nitrogen found is the result of the accumulation of the greater part of the amino acids formed in the medium. In general, the amount of ammonia nitrogen increases with the progress of the decomposition of protein. Though in the case of *Mucor racemosus*, judging from the amount of ammonia formed, the decomposition of protein is inferior to that in the case of *Asper. glaucus*, the sum of the amino and ammonia nitrogen values is greater in the former than in the latter. It is therefore evident that the mere

determination of the amount of either amino or ammonia nitrogen for the calculation of the grade of protein decomposition is irrelevant. This fact will be more clearly shown by the following experiment with peptone.

### 2. WITTE's peptone.

In this experiment WITTE's peptone was used in a concentration of two per cent. The results are shown in Table II.

TABLE II.  
The decomposition of 2% WITTE's peptone.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	Gain in the sum of amino- and ammonia-N.	pH
		mg.	mg.	mg.	
1	Control	23.1	13.0	—	5.53
2	<i>Bac. albolactis</i>	95.7	85.8	145.4	7.67
3	<i>Bac. graveolens</i>	61.7	46.2	71.8	7.22
4	<i>Bact. gastricum</i>	84.9	111.0	159.8	8.69
5	<i>Bact. coli-anindolicum</i>	37.0	48.5	49.4	7.64
6	<i>Bact. diffusum</i>	31.5	25.2	20.6	6.76
7	<i>Asper. glaucus</i>	50.6	62.1	76.6	9.18
8	<i>Penic. glaucum</i>	68.0	91.8	123.7	8.47
9	<i>Mucor. mucedo</i>	66.6	47.0	77.5	7.43
10	<i>M. racemosus</i>	98.0	29.8	91.7	6.89
11	<i>Rhizopus nigricans</i>	66.1	58.9	88.9	8.12

By *Bac. albolactis*, *Bact. gastricum*, *Penicillium*, *Aspergillus* and *Rhizopus* the peptone is attacked in a similar degree to that in the case of casein.

### 3. Glycylglycine.

The glycylglycine used was in the commercial form manufactured by KAHLBAUM, its constitutional formula being  $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{HN} \cdot \text{CH}_2 \cdot \text{COOH}$ . In the case of glycylglycine only its primary amino nitrogen, not united with the carboxyl group, gives a reaction by VAN SLYKE's method, so that the theoretical amount of measurable amino nitrogen in one gram should be 106.04 mg. The results obtained are shown in Table III.

Table III shows that it is not possible for *Bac. graveolens*, *Bac. albolactis*, *Bact. gastricum* and *Mucor mucedo* to grow at all in the medium, and that no decomposition of glycylglycine takes place. As shown by the former experiments (Table I and II) *Bac. albolactis* and *Bact. gastricum* strongly attack casein and peptone, while *Bact. coli-anindolicum*, and *Bact. diffusum* which are able to decompose glycylglycine do not attack those substances or only very slightly.

TABLE III.  
The decomposition of 1% glycylglycine.

No.	Micro-organisms	Amino-N in 100 c.c.*	Ammonia-N in 100 c.c.	Gain in the sum of amino- and ammonia-N.	pH
		mg.	mg.	mg.	
1	Control	105.2	0.3	—	5.43
2	<i>Bac. albolactis</i>	105.0	0.3	—	5.45
3	<i>Bac. glaveolens</i>	104.9	0.3	—	5.42
4	<i>Bact. gastricum</i>	105.1	0.4	—	5.44
5	<i>Bact. coli-anindolicum</i>	96.1	24.0	14.6	7.30
6	<i>Bact. diffusum</i>	97.9	21.1	14.5	7.41
7	<i>Asper. glaucus</i>	102.8	51.4	48.7	8.25
8	<i>Penic. glaucum</i>	59.5	84.3	33.3	8.68
9	<i>Mucor mucedo</i>	104.9	0.4	—	5.45
10	<i>M. racemosus</i>	109.1	8.3	11.9	6.90
11	<i>Rhizopus nigricans</i>	107.3	13.0	14.8	7.02

\*) According to VAN SLYKE's (Journ. Biol. Chem., Vol. 9, P. 185-204, 1911) the glycylglycine and glycocoll react abnormally with nitrous acid, all the analyses being somewhat higher than the theoretical expectation. The values noted in Table III and IV are those corrected for this abnormality.

TABLE IV.  
The decomposition of 1% glycocoll.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	pH
		mg.	mg.	
1	Control	181.8	0.5	5.60
2	<i>Bac. albolactis</i>	181.1	0.6	5.60
3	<i>Bac. glaveolens</i>	181.7	0.4	5.60
4	<i>Bact. gastricum</i>	181.6	0.5	5.56
5	<i>Bact. coli-anindolicum</i>	153.8	25.2	7.34
6	<i>Bact. diffusum</i>	173.0	7.4	6.09
7	<i>Asper. glaucus</i>	139.0	39.0	8.02
8	<i>Penic. glaucum</i>	121.2	58.8	8.25
9	<i>Mucor mucedo</i>	181.5	0.6	5.60
10	<i>M. racemosus</i>	148.2	29.0	7.58
11	<i>Rhizopus nigricans</i>	121.7	56.4	8.20



## 4. Glycocoll.

The constitutional formula of glycocoll is  $\text{CH}_2(\text{NH}_2)\text{COOH}$ , so that it contains theoretically 186.65 mg nitrogen in one gram. The results are given in Table IV.

Table IV shows that glycocoll can be decomposed by *Bact. coli-anindolicum*, *Bact. diffusum*, *Aspergillus*, *Penicillium*, *Mucor racemosus*, and *Rhizopus*, that is, only by the organisms which can decompose glycylglycine. As to the amino nitrogen, in the case of mono-amino acids, such as glycocoll and alanine a decrease in amount would naturally be expected, if they were broken up by the organisms. The sum of the measurable amino- and ammonia nitrogen values stand approximately the same as that of the control.

5.  $\alpha$ -alanine.

The constitutional formula for  $\alpha$ -alanine is  $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$ , therefore, it contains theoretically 157.29 mg nitrogen in one gram. The results are given in Table V.

TABLE V.  
The decomposition of 1%  $\alpha$ -alanine.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	pH
		mg.	mg.	
1	Control	157.1	0.3	5.07
2	<i>Bac. albolactis</i>	156.8	0.3	5.08
3	<i>Bac. glaveolens</i>	131.7	21.2	7.28
4	<i>Bact. gastricum</i>	130.6	25.0	7.33
5	<i>Bact. coli-anindolicum</i>	96.8	56.1	7.74
6	<i>Bact. diffusum</i>	156.7	0.5	5.09
7	<i>Asper. glaucus</i>	119.1	34.0	7.53
8	<i>Penic. glaucum</i>	50.1	100.2	8.33
9	<i>Mucor mucedo</i>	129.5	27.3	7.39
10	<i>M. racemosus</i>	136.5	13.3	7.18
11	<i>Rhizopus nigricans</i>	83.1	68.7	7.86

It may be seen in the above table that alanine is attacked by the majority of organisms with the exception of *Bac. albolactis* and *Bact. diffusum*. In the case of *Penicillium*, *Rhizopus* and *Bact. coli-anindolicum* particularly, the decomposition is most remarkable.

c. *Experiments in the Medium pH 3.3.*

In this acidic medium peptone and glycoll are decomposed only by *Penicillium*, *Aspergillus* and *Rhizopus*. (Table VI and VII).

TABLE VI.\*)

The decomposition of 2% WITTE's peptone in an acidic medium.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	pH
1	Control	mg. 30.3	mg. 12.7	3.25
2	<i>Asper. glaucus</i>	117.0	94.6	8.18
3	<i>Penic. glaucum</i>	57.0	119.3	8.29
4	<i>Rhizopus nigricans</i>	37.5	93.9	8.05

TABLE VII.\*)

The decomposition of 1% glycoll in an acidic medium.

No.	Micro-organisms	Amino-N in 100 c.c.	Ammonia-N in 100 c.c.	pH
1	Control	mg. 179.3	mg. 0.3	3.37
2	<i>Asper. glaucus</i>	142.5	37.0	7.84
3	<i>Penic. glaucum</i>	125.5	52.5	8.38
4	<i>Rhizopus nigricans</i>	126.3	51.5	8.30

\*) The other organisms not recorded in these tables do not attack the peptone and glycoll in this acidic medium, so that in their cases there are no results to be given.

DERNBY found that the optimum acidity for the decomposition of gelatine or peptones by *Bac. subtilis*, *Bac. pyocyaneus*, *Bac. proteus* and some others is nearly at pH 6-7, and the growth and the action of the enzymes of organisms are both limited to the same range of acidity, that is to pH 4-9. Similar results were also obtained by DERNBY and BLANC in the experiment with some species of *Clostridium*. The bacteria and fungi used in my work, with the exception of *Aspergillus*, *Penicillium* and *Rhizopus*, are in their action on proteins and in their degradation products similar to those with which DERNBY's experiments were carried out.

Looking through the results of all the experiments above-mentioned, *Bact. gastricum*, which hydrolyzes casein and peptone with great ease are unable to attack the amino-acids used, while in the case of *Bact. coli-*

*anindolicum* these effects are reversed, glycooll and alanine being easily attacked, and casein and peptone slightly hydrolyzed. In cases of fungi, such as *Penicillium*, *Aspergillus* and *Rhizopus*, the protein, peptides and amino acids used are easily decomposed both in a strong and weak acidic medium; this effect being especially noticeable in *Penicillium* and *Aspergillus*. *Mucor mucedo* and *M. racemosus* do not attack the amino acids used or only very slightly. In general they are only capable of decomposing some nitrogenous compounds under the optimum conditions, and their preference for some compounds to others is clearly demonstrated.

The fact, however, that the majority of organisms isolated from insectivorous plants can not grow in a medium of about pH 3.3. may lead to the question whether they really do take part in the decomposition of insect bodies by insectivorous plants or not. In my view, insect bodies are probably first attacked by those organisms which are capable of growing in a strong acidic medium, while the others, after the acidity of the medium has changed so as to be weak, come to take an active part in the decomposition.

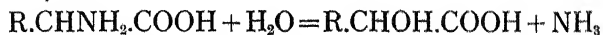
In consideration of the characteristics of the proteolytic enzyme in leaves of *Drosera* and in pitchers of *Nepenthes*, as shown by CLAUTRIAU (1900), VINES (1901), and OKAHARA (1930), we are directed to the conclusion that the micro-organisms, if not the enzymes of other kind should be proved to exist in the fluid of those plants at all, may be considered to take a cooperating part in the decomposition of insect bodies, so far as the nutrition of those plants is concerned.

d. *On the Process of Decomposition of Amino Acids by Micro-organisms.*

EHRlich reported that the formation of higher alcohols from amino acids was a single case of hydrolysis causing the liberation of carbon dioxide and ammonia, and that the alcohols formed had one carbon atom less than the amino acids.

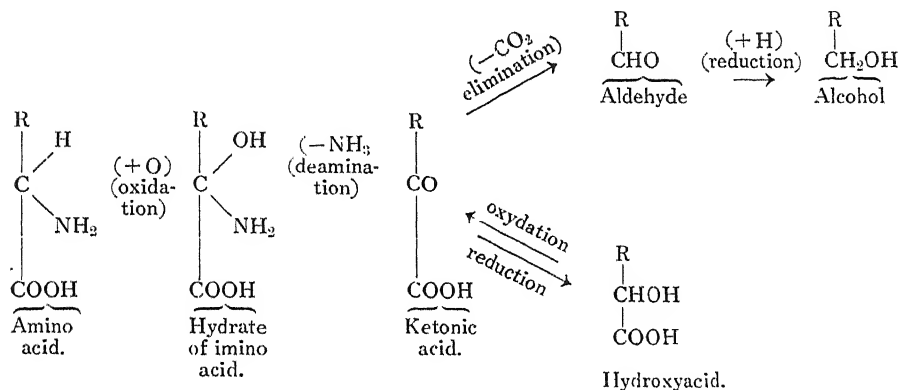


And also, according to him, the formation of  $\alpha$ -hydroxyacid by the hydrolytic deamination of amino acid, replacing  $NH_2$ -group with OH, seems probably to be true.

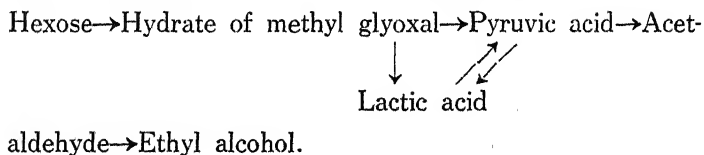


In the study of the decomposition of phenyl amino acetic acid to *p*-oxyphenyl ethyl alcohol by yeast, NEUBAUER and FROMHERZ (1910-11) reported that the formation of higher alcohols from amino acids passes through more complex intermediate stages than is suggested by EHRlich; and that  $\alpha$ -

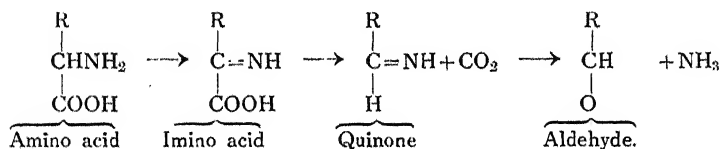
hydroxyacid may not be produced by direct hydrolytic deamination, but rather by the reduction of  $\alpha$ -ketonic acid. They represented the process of alcoholic fermentation by the following scheme:



If this idea is applied in the case of alanine, the process of its alcoholic fermentation comes to have a remarkable resemblance to that of the transformation of hexoses, as postulated by NEUBERG:



WIELAND and BERGEL (1924), however, objected to the suggestion of NEUBAUER and FROMHERZ on the ground that the amino acid is decomposed through the form of quinone to aldehyde in the following way:



In the biological transformation of amino acid, however,  $\alpha$ -hydroxy and  $\alpha$ -ketonic acids are generally formed. The process of formation of these acids cannot be explained by WIELAND and BERGEL's scheme, though their suggestion may surely be true from the standpoint of pure chemistry.

To throw some light on this question the following researches were carried out. The culture media used in these experiments were prepared by the addition of 7.5 g glycocoll or alanine to each 500 c.c. of the liquid

medium above-mentioned, which contained only inorganic salts, and was inoculated with *Penicillium glaucum*, after it had been left in the thermostat for 20 days at about 30°.

1) The decomposition of glycoll.

The filtrate of the culture liquid was distilled, and the tests for methyl alcohol, formaldehyde, formic and acetic acids were made with the distillate, but the reactions turned out negative. Then the tests for glyoxylic acid were carried out with the distillate as follows:

i) The liquid to be tested was mixed with indol, skatol or tryptophane, then concentrated sulphuric acid was added being poured down along the side of the tube. A reddish violet ring was distinctly formed at the junction of the liquids.

ii) The liquid to be tested was treated with an excess of calcium hydroxide on the water-bath, then filtered off. The filtrate was cooled, and characteristic crystals of calcium glyoxalate in the form of white prisms were readily collected.

It is well known that many chemicals, e. g. acetic acid, alcohol, acetone etc., in an ordinary state of purity contain a trace of glyoxylic acid, so that it is necessary to test this acid in a culture liquid of the same composition, inoculated but with no organism as a control. Such a liquid gave no reaction for glyoxylic acid.

Thus the production of glyoxylic acid by the micro-organisms is very probable. Moreover, the positive results of the general reaction for hydroxy-acid in the distillate of the culture liquid have led me to the idea that glycollic acid may also be produced, though the isolation of it failed.

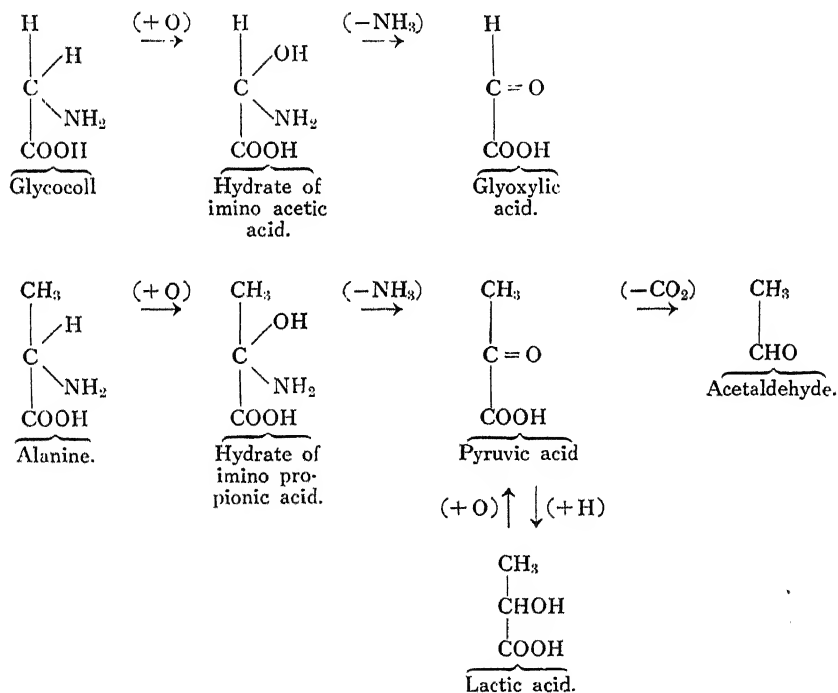
2) The decomposition of alanine.

The tests on acetaldehyde, acetic and propionic acids were carried out with the distillate of the culture liquid, but the results were negative. The residue in the flask which gave a strong positive iodoform reaction in the cold was condensed on the water-bath. From the characteristic odour of the syrup the presence of pyruvic acid was presumed. The syrup was distilled fractionally in a vacuum at about 65°/12 mm. With the distillate the following tests were carried out: the reduction of ammoniacal silver nitrate, the red colour formation in the presence of ferric-chloride dissolved in ether, the iodoform reaction in the cold, and the blue colour reaction by the addition of nitro-prusside sodium and ammonia. All these tests gave positive results. Therefore pyruvic acid may be assumed to have been produced in the culture liquid.

Further, to detect lactic acid in the culture liquid, the latter was heated

on the water-bath, and oxalic acid was added to precipitate calcium salt. The liquid was filtered off and evaporated to a syrup. This syrup was extracted with ether, evaporated, and then dissolved in water. The aqueous solution was added with baryta, the excess of which was treated with carbon dioxide. The mixture was boiled to decompose the barium carbonate, filtered, and the filtrate was treated with zinc carbonate. Crystals in the form of white prisms were isolated. With these crystals, FLETCHTER and HOPKIN's and DENIGÈ's reactions for lactic acid were tested. The result was positive.

In consideration of the formation of glyoxylic acid from glycocoll and of the lactic and pyruvic acids from alanine, these amino acids, in accordance with the NEUBAUER and FROMHERZ scheme, seem to be transformed, respectively, in the following ways:



ACKLIN (1929) said that the transformation of lactic acid by *Penicillium glaucum* proceeds through pyruvic acid to acetaldehyde. So it may not be impossible to postulate the formation of acetaldehyde in the decomposition of alanine under the action of micro-organisms, though the detection

of it was unsuccessful.

The results obtained in this study point to the conclusion that, though the main decomposition products of amino acid may be different for different organisms, e. g. for acetic or propionic acids fermenting bacteria, the main process of transformation will be ruled by NEUBAUER and FROMHERZ' scheme, as in the case of hexoses which are ruled by that of NEUBERG.

### III. SUMMARY.

1. The following micro-organisms have been isolated from the leaves of *Drosera rotundifolia* and the pitchers of *Nepenthes mirabilis*, viz. *Bac. albolactis*, *Bac. graveolens*, *Bact. gastricum*, *Bact. coli-anindolicum*, *Bact. diffusum*, *Mucor mucedo*, *M. racemosus*, *Aspergillus glaucus*, *Penicillium glaucum*, *Rhizopus nigricans*, *Actinomyces* and yeast.

2. The degree of decomposition of casein, WITTE's peptone, glycylglycine, glycocoll and alanine by these organisms, with the exception of *Actinomyces* and yeast, has been investigated in estimating the amino and ammonia nitrogen values.

3. The experiments were carried out in two kinds of acidic medium, one pH 5-6, and the other pH 3.3. In the culture medium pH 5-6 the majority of micro-organisms isolated are capable of decomposing the nitrogenous compounds above-mentioned. In the medium pH 3.3, WITTE's peptone and glycocoll were decomposed only by *Aspergillus glaucus*, *Penicillium glaucum*, and *Rhizopus nigricans*.

4. The organisms have mutual preferences in the decomposition of proteins and their degradation products. *Bac. albolactis* and *Bact. gastricum* which attack casein and peptone easily could not or only slightly decompose the amino acids used. *Bact. coli-anindolicum* slightly attacks casein, but actively decomposes alanine. Two types of organisms may cooperate in the decomposition of protein to ammonia.

5. By the action of *Penicillium glaucum* glyoxylic acid is probably formed from glycocoll, and lactic and pyruvic acids from alanine. And the process of the transformation of amino acids seems to follow the NEUBAUER and FROMHERZ' scheme.

6. Looking through the results obtained it is likely that in the whole process of decomposition of protein in insectivorous plants the enzymes secreted by the plants themselves may take a leading part, but the micro-organisms, such as are isolated from those plants, may also cooperate in the completion of the process.

In conclusion, I wish to thank Prof. Dr. Y. YAMAGUTI for his help

and the Government of the South Sea Islands for the kindness shown in supplying me with ample living materials of *Nepenthes*.

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# THE COLONY OF THE LIMPET (*ACMAEA* *DORSUOSA* GOULD)<sup>1)</sup>

By

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(With 9 text-figs.)

(Received Feb. 10, 1933)

## INTRODUCTION.

Among the colony forming species of Gastropods, *Littorina*, *Purpura* and *Acmaea dorsuosa* are seen commonly in Mutsu Bay. In my previous paper (ABE, 1931) I have reported some accounts on the formation as well as dissolution of a colony by the limpet, *Acmaea dorsuosa*, and I will now present further observations carried out since spring of 1931. The purpose of the study was to throw some light on the point of why and how the limpet forms and desolves the colony.

Observations have been done at the Asamushi Marine Biological Station. While the observations were being carried out, Prof. Dr. S. HATAI informed me in many ways. I wish to express my hearty thanks to him and to the other members of the station for their kind assistance.

## FORMATION AND DISPERSION OF COLONY.

Limpets were marked by painting the tops of their shells by red enamel in one colony and by white enamel in the other in summer, and their movements were closely observed on the rocky cliff of Hadakajima, near the station.

### a) The Variation as to the Time of Dispersion.

1. *Individual variation.* Although *Acmaea dorsuosa* dissolves the colony during about the middle of September to the beginning of October, yet there is considerable variation as to the time of dispersion according to the individuals even in one home, as shown in Table 1.

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<sup>1)</sup> Contributions from the Marine Biological Station, Asamushi, Aomori-Ken, No. 101.

TABLE I.  
Individual variation on dispersion.

Colony Date, 1931	No. 1	No. 2	No. 3
Sept. 8th.	no dispersion occured (21)	no dispersion occurred (21)	34 in the home (47)
„ 22nd.	dispersion begun	„	„
„ 26th.	7 in the home	„	„
„ 30th.	5 „	„	25 in the home
Oct. 2nd.	2 „	dispersion begun	11 „
„ 7th.	2 „	12 in the home	11 „
„ 11th.	3 „	„	7 „
„ 13th.	completed	8 „	1 „
„ 16th.	0	2 „	completed
„ 21st.	0	completed	0
„ 24th.	0	0	0

In colony No. 1, some limpets had already dispersed on Sept. 22nd and yet 3 limpets still remained in the home until Oct. 11th, showing at least a 20 day difference between the earliest and the latest. Colony No. 2 shows a 15 day difference, and colony No. 3, a 35 day difference. So it is clear that the limpets show individual variation as to the time of dispersion.

2. *Time of dispersion in relation to age.* Relation between time of dispersion and age of limpet is shown in Table II.

TABLE II.

Time of dispersion in relation to the ages of limpets.

No. of colony	Age in years	Sept. 27th.	Oct. 8th.	Oct. 12th.	Oct. 28th.
I	1	0	0	0	2*
	2	8	5	3	4
	3	9	2	2	0
	4	2	2	2	2
	5	13	8	3	0
	6	9	1	1	0
	7	0	0	0	0

No. of colony	Age in years	Sept. 27th.	Oct. 8th.	Oct. 12th.	Oct. 28th.
II	1	29	7	6	20*
	2	50	23	6	12*
	3	14	3	2	1
	4	16	16	5	0
	5	18	4	1	0
	6	0	0	0	0
	7	0	0	0	0
III	1	0	0	0	0
	2	7	9*	1	3*
	3	2	0	0	0
	4	1	0	0	0
	5	4	1	0	0
	6	2	2	0	0
	7	2	0	0	0

\* Younger limpets frequently migrate even in the period of dispersion.

From Table II, we can see clearly that younger limpets less than 3 years of age disperse comparatively later than older ones of more than 4 years of age. Table III shows the individuals in the home and out of the home.

TABLE III.

Limpets in and out of the three colonies combined.  
(Sept. 27th, 1932).

Age in years	1	2	3	4	5	6	7	8	9
Total no. of inds. in these homes	0	10	12	4	18	10	1	0	1
No. of inds. which dispersed out but are found close to the homes.	0	1	0	6	16	10	6	6	3

3. *Time of dispersion in relation to the location of the colonies.*  
Comparing the limpets in one colony to those in the other, one notices clear difference as to the time of dispersion (Table IV).

From Table IV, we can see that the limpets in the home which faces the west dissolves the colony earlier than those facing the east, influenced probably by waves, since west and north-west winds blow strongly and most frequently in the cold season while east and north-east winds blow but only occasionally, and since the rock shore of Hadakajima faces both west and north-east, those homes facing the west receives the wave influence most strongly. It was noticed that if two colonies are formed, one above

TABLE IV.

Directions of the colonies in their relation to the time of dispersion.

Colony Date 1932	No. 1 120° NE	No. 2 210° NNW	No. 3 185° NNNW	No. 4 65° WNW
Sept. 2nd.	no dispersion occurred	no dispersion occurred	no dispersion occurred	no dispersion occurred
" 19th.	"	"	dispersion begun	dispersion begun
" 23rd.	"	dispersion begun	1st stage	1st stage
" 29th.	dispersion begun	1st stage	"	"
Oct. 2nd.	1st stage	"	2nd stage	2nd stage
" 8th.	"	2nd stage	"	completed
" 12th.	2nd stage	"	completed	winter life
" 21st.	completed	"	winter life	"
" 28th.	winter life	completed	"	"

1st stage indicates that about 1/3 part of the limpets are dispersed. 2nd stage indicates that the majority of the limpets are dispersed. Winter life indicates that the limpets moved upward from the home and began winter life.

the other, the dispersion begins earlier in the lower colony than in the upper colony. (See Fig. 4, colonies E and C)

#### b) Vertical Dispersion.

1. *Measurement of dispersed distance.* Although the time of dispersion varies considerably according to the individuals but ultimately all the limpets scatter away from the home. The direction of scattering may be conveniently divided to two dimensions, vertical dispersion and horizontal dispersion.

The distance of dispersion can not be accurately measured because the face of the rock is not only uneven but inclines about 46 to 59 degrees on horizontal plane, though so far as the degree of inclination is concerned, it can be calculated from the relation  $a \sin \theta$ . The measurements taken along the inclined surface show utmost about 2 meters above and 1.6 meters below the home level.

2. *Relation of vertical dispersion to temperature.* The vertical dispersion of the limpets in two colonies, consisted of young limpets of two years of age and of older limpets of 6 or 7 years of age as shown in Fig. 1.

In Fig. 1, a middle horizontal line shows a level of the uppermost edge of the home. Heavy line AB shows the period during which the limpets recolonize in 1931. At B, or on about Sept. 15th, 1931, dispersion of the colony began and some of them dispersed upward from the home and others downward, exact positions occupied during subsequent dates by those traversed from the home level are indicated by the zigzag courses. At C, or on about April 10th, 1932, the limpets began to recolonize in 1932. Similar as at B, D, or on about Sept. 20th, is a point of dispersion in 1932.

On B and D, where the limpets began to disperse, we notice a fall of atmospheric temperature under  $15^{\circ}\text{C}$ , which continues to fall gradually during the subsequent days. As for dispersion, although we notice the fall of temperature below  $15^{\circ}\text{C}$  on Sept. 5th, the limpets did not show dispersion, due probably to the rise of temperature in subsequent days. Similarly on D, the atmospheric temperature fell below  $15^{\circ}\text{C}$  on Sept. 13th and again on 19th, after which it fell gradually and continuously. Some of the limpets began to disperse on about the 13th though the majority began after 19th.

The dispersion of the limpet or dissolution of the colony seems to me to be related to the steady and continuous fall of atmospheric temperature below at  $15^{\circ}\text{C}$  in its minimum temperature during late autumn season.

Examining the movements of limpets from B to C, it will be also noticed that after dissolution till towards the end of Oct., the individuals which moved upward from the home are more numerous than those which moved downward. But after the 20th of October the limpets dispersed downward gradually and after Nov. 20th almost all of the limpets began to crawl downward, and the majority of them were seen one meter above the home level.

On the other hand the re-formation of the colony seems to have begun during the spring season when the atmospheric temperature tends to rise steadily and continuously till about  $13^{\circ}\text{C}$  (in its maximum temperature) or above. For instance the first ten days of April in 1932, the atmospheric temperature rose to about  $13^{\circ}\text{C}$  and the limpets appear ready to form the colony, as indicated by the presence of the limpets on the rock face of Hadakajima, and in reality all of the limpets recolonized shortly after.

3. *Wave in relation to dispersion.* The colony of the limpet is always formed above high-water mark, and since they can not creep without the aid of splashing water, the waves are seemingly important in their dispersion. In Fig. 1, gales more than 8 or 9 meters per second are indicated

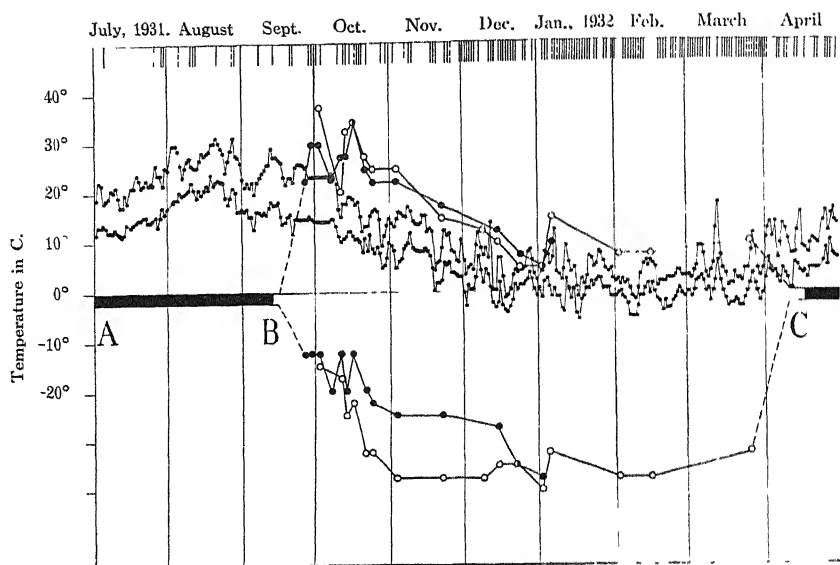


Fig. 1. Vertical dispersion of the limpet.

by short lines drawn perpendicular from the top. Winds of 8 meters per second accompanies white-crested waves which wash over the rock face where the limpets are.

Look upon B in Fig. 1 or on Sept. 5th and 6th, the minimum atmospheric temperature was about  $13^{\circ}\text{C}$ , but no stronger winds were recorded and no dispersion of limpets was noted, but on 13th., 12 meters gale and on 20th and 21st, 13–15 meters gale blew and the dispersion of limpets was noted in those days. On D or on Sept. 7th and the following several days, 10 to 12 meters gale was recorded and there we noted the dispersal of limpets.

I may mention here that the limpets are found under snow without showing apparent injurious effect.

4. *Relation of age to vertical dispersion.* I have made a frame of one meter in breadth and 1.4 meters in length, which were divided into 7 partitions, by strings which were fastened 20 centimeters apart. With this frame I have determined the position of limpets on the rock face, away from the level of the uppermost edge of the home. Limit of measurement is 6 meters from the east end to the west end of the rock of Hadakajima. An example of the determination is shown in Table V.

Table V shows that the range of vertical dispersion varies with the age of limpet; that is, limpets younger than 3 years of age are almost

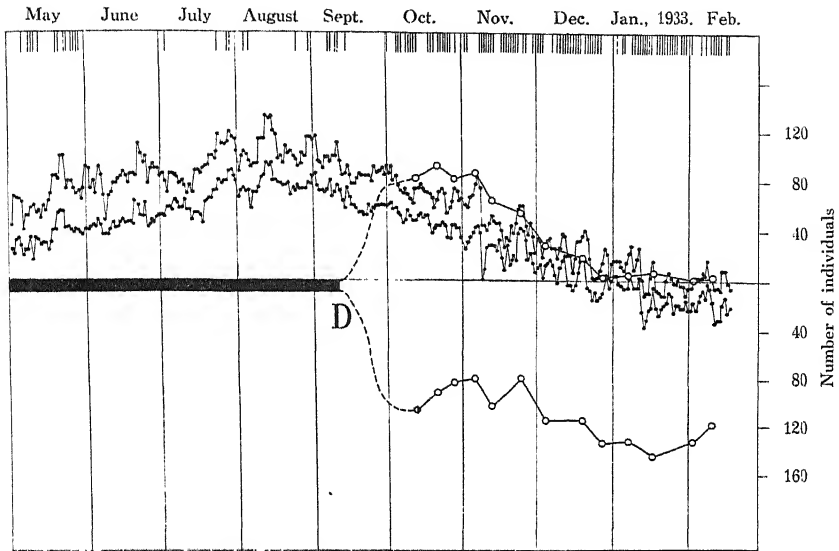


TABLE V.  
Vertical distribution of the limpet.

Age in years Height in meters	1	2	3	4	5	6	7	8	9	Total
2 M				1		1		2		4
1.8				2						2
1.6				3	3	1				7
1.4				6	6				1	13
1.2			1	1	2					4
1 M				4	1		1			6
0.8				4						4
0.6				1	3					4
0.4		1		5	2	1				9
0.2		8	2	2			1			13
Home level										
0.2	3	20	7	7	6					43
0.4	3	16	3	5	4	1				32
0.6	3	8	1	4	3	1				20
0.8			2	2	1					5
1 M				1						1
Total number										167

Hadakajima, Nov. 12th, 1932.



always below the home level, and those above 4 or 5 years of age occupy upper a higher position from the home, while the limpets of 1 or 5 years of age are dispersed both above and below.

Now dividing the limpets into two groups, the younger group less than 3 years of age and the older over 4 years of age, we notice their respective dispersion as shown in Fig. 2 and Fig. 3.

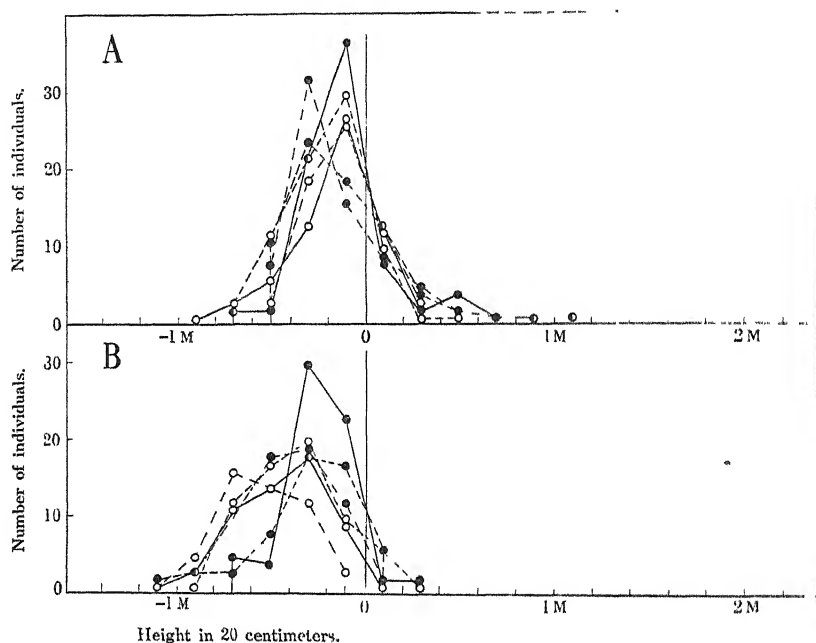


Fig. 2. Vertical distribution of the young limpet.

A: ●—● Oct. 12th.	B: ●—● Dec. 4th.
●—● „ 21st.	●—● „ 19th.
●—● „ 28th.	●—● „ 27th.
○ ○ Nov. 5th.	○ ○ Jan. 7th.
○—○ „ 12th.	○—○ „ 17th.
○—○ „ 24th.	○—○ Feb. 2nd.

As is shown in Fig. 2, the frequency distribution of the young limpet is almost symmetrical; that is, the number of limpets diminishes as the distance from the home level increases in both the periods in A and B, though the range as well as the position of maximum frequencies is not identical. We notice in A or in the first three months after dispersion, the limit of range is practically one meter above and below the home level and its maximum point thus lies only within 40 centimeters below

the home level as is shown in Fig. 2. But in the later days or in B, the position of maxima moves farther below 40 centimeters or approximately lies within 60 centimeters below it.

The vertical distributions of the older group in A and B are shown in Fig. 3.

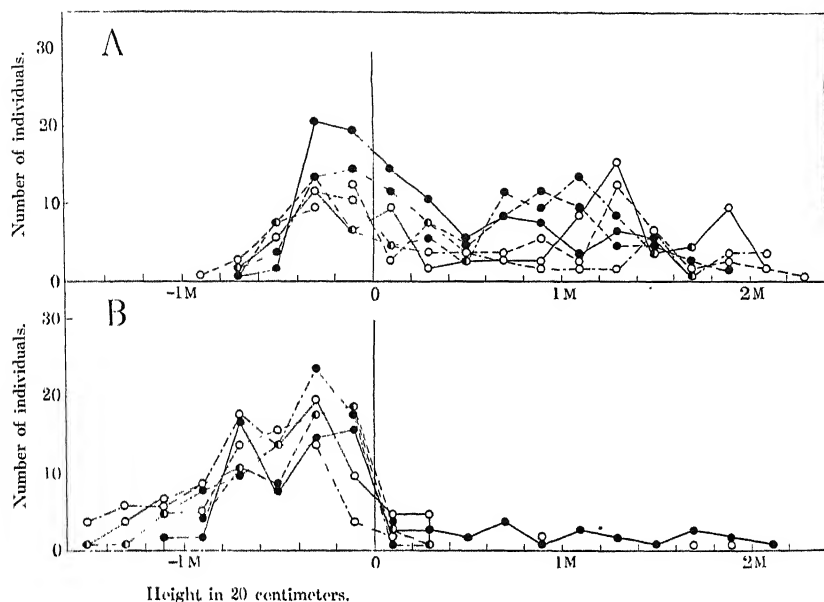


Fig. 3. Vertical distribution of the old limpet.

A: ●—● Oct. 12th.	B: ●—● Dec. 4th.
●—● " 21st.	●—● " 19th.
●—● " 28th.	●—● " 27th.
○—○ Nov. 5th.	○—○ Jan. 7th.
○—○ " 12th.	○—○ " 17th.
○—○ " 24th.	○—○ Feb. 2nd.

The range of distribution is wide. In A it lies between about 2 meters above and one meter below the home level, while in the later days or in B, the range changes from 40 centimeters above to 160 centimeters below the home level, we thus notice that seasonal variations of the frequency distribution are very conspicuous, as will be seen from the respective positions occupied at different periods. That is 84:106 ( $\pm 0.79:1.0$ ) on Oct. 12th, 29:124 ( $\pm 0.23:1.0$ ) on Dec. 4th and 7:144 ( $\pm 0.05:1.0$ ) on Jan. 17th.

So it is clear that the limpets in the earlier period of dispersion is upwards and yet after the beginning of December, it retreats downward.

Beside this, it seems to worth noting that several limpets in B move farther below the level of 160 centimeters from the home level than the lowest occupied by the limpets in A.

Here I wish to point out that *Acmaea dorsuosa* makes a home at different levels (Fig. 4, a) from other animal associations which also inhabit a position closer to the water level such as *Chthamalus challenger* HOEK and *Mytilus crassitesta* LISCHKE,\* but after dissolution of the colony they are often found associated among those of the later.

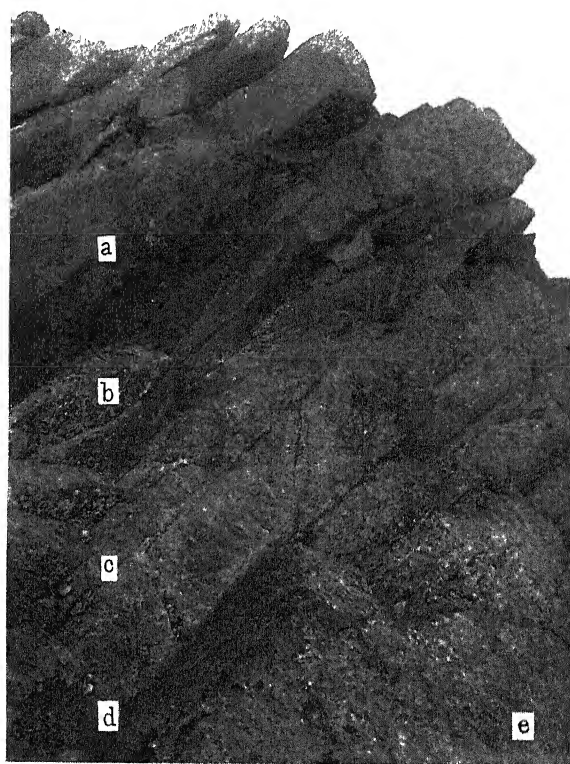


Fig. 4. Rock face at lowtide, showing the association of the limpet.

- a) *Acmaea dorsuosa* GOULD.
- b) *Chthamalus challenger* HOEK.
- c) *Septifer virgatus* (WIEGMANN)\*
- d) *Motilus crassitesta* LISCHKE.\*
- e) *Asterias rollestoni* SLADEN.

(photographed on Sept. 24, 1931)

\*On the identification of the species name of the shells above quoted, I am much indebted to Mr. SHICHIHEI NOMURA. Here I wish to express my sincere thanks to him.

## c) Horizontal Dispersion and Home.

1. *Horizontal dispersion and reformation of new colony.* The limpet disperses horizontally as well vertically as already mentioned. On the time of dispersion, one part of the limpets disperse to the westward and the other to the eastward, and the maximum distances traversed to both are almost the same; that is, 6 meters away from the original home.

In one home it was found that the number of limpets which dispersed to the eastward to the ones which dispersed to the westward were 15:8 respectively in the first month after dispersion. But the limpets which formed their home one meter to the eastward from the first home mentioned already, it was 7:13 respectively. In both cases nearly  $\frac{1}{3}$  of the limpets occupied the positions within both directions about 1 meter from the home.

The limpet shows homing habit, but it is very doubtful that the limpet which disperses several meters away from the home may ever return to the original home in the next spring. To test this point I have examined the individuals in many reformed new colonies at spring of the following year, and the results are shown in Fig. 5.

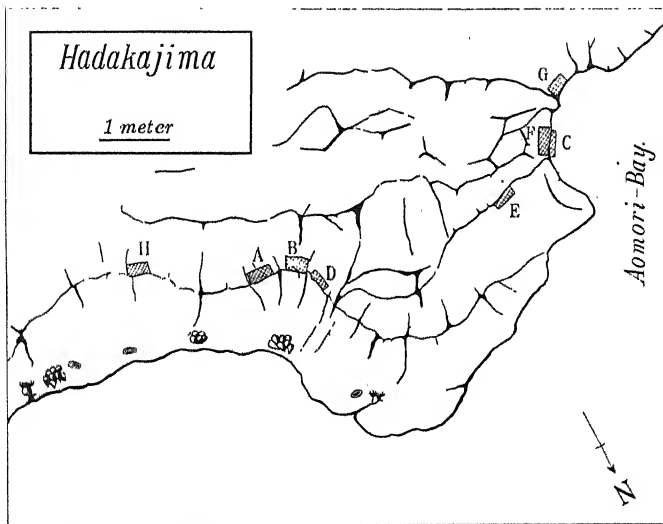


Fig. 5. Rock shore of Hadakajima, showing the colonies of *Acmaea dorsuosa* GOULD in 1932.

In Fig. 5, the colonies lettered A, B and C are the places where the colonies were formed in 1931, and the individuals there found were 25 in A, 19 in B and about 250 in C. In the spring of 1932, colonies were

reformed on the same positions but the individuals which entered into the formation of these homes involved many newcomers. At the same time entirely new colonies were formed on D, E, F, G, and H, involved several limpets which were found in A, B and C in 1931. Furthermore the limpets in the home B in 1931 are seen A, B, C and D in 1932. The migration of limpets just mentioned are diagrammatically shown in Fig. 6.

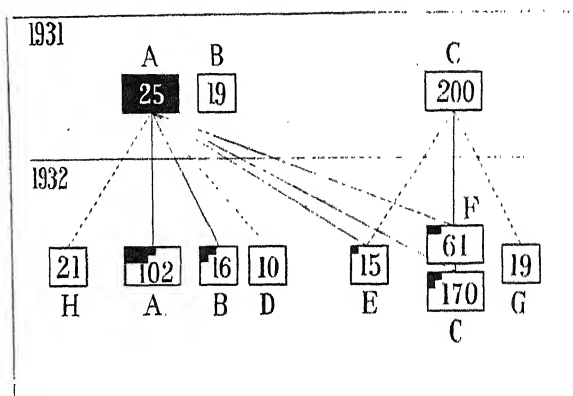


Fig. 6. Re-formation of the colony.

It seems therefore that the colony is reorganized yearly with addition of new members. Although it is a fact that one part of them returns exactly to their original home of the former year, but whether or not they return to the original home by mere accident or by homing habit can not yet be definitely stated.

2. *On the home.* The limpets usually reform their colony on the place where the homes were formed in the former year, but often a new home is formed either only within 15 to 20 centimeters away from the old home or at entirely different places where no old homes are to be found in their neighborhood. It is also the case that the homes of previous year are cast away and are occupied for only one year.

As for the scar, there are scars clearly printed or almost not printed, according to the properties of the rock. In Fig. 7, we can see scars printed clearly. This rock is quartz-trachyte and the scar was marked only within 5 months.

The scar is not extinguished in a year and new scars are printed over that of the former year, so there are seen double or even triple scars. The scar may be printed by some kind of an acid secreted by the limpet and also by friction of the edge of the shell, but I do not yet know of the nature of the secretion.

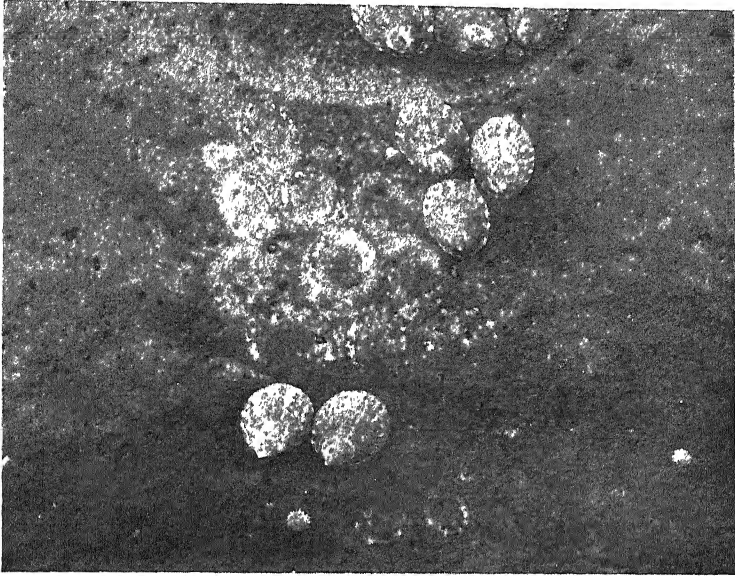


Fig. 7. Scars marked by the limpet.  
(photographed on Oct. 2nd, 1931 at Hadakajima, No. 1. in Table IV.)

## II. EXPERIMENTS ON THE MODE OF COLONY FORMATION.

*Acmaea dorsuosa* exhibits the habit of colony formation in the laboratory as in nature. I have therefore followed carefully in the laboratory the formation and dissolution of a colony and will present some of the results so far attained.

### a) Method.

A middle sized cylindrical glass vessel, diameter 30 cm. and height 25 cm., was filled with sea-water up to 10 cm. below the uppermost edge. The water is constantly running. And the wall of the mouth was eight-sected, and each of the section was numbered from 1 to 8. Sixteen limpets were placed on the inner wall, one along the middle line of each pertition and other one on the lines, 15 cm. under the water surface. The limpets thus placed on the wall begin to creep upward by negative geotropism (ABE, 1931) and nearly reach the water surface.

On the next morning the positions of the limpets were marked from the outside of the glass vessel and then were printed on a plotting-paper. Such processes continued 8 to 10 days in each experiment.

### b) Experiments.

Exp. 1 On the limpet before dispersion.

The first experiment began on August 31st, using the limpets which were collected in one colony of Yunoshima and the observation was continued till near the end of September. These the limpet formed a colony even in the laboratory and did not disperse during the period of observation. All of the limpets so far examined showed the habit of colony formation as is shown in Table VI.

TABLE VI.  
Colony formation of the limpet before dispersion.

Exp.	No. 1. (August, 31st.) 28.9°C-18.0°C						No. 2. (Sept. 6th.) 28.0°C-17.6°C						No. 3. (Sept. 21st.) 23.4°C-13.6°C					
Days after Section	1	2	3	4	5	Colony	1	2	3	4	5	Colony	1	2	3	4	5	Colony
1	0	0	0	0	0	—	0	0	0	0	0	—	7	10	10	9	10	+10
2	5	4	5	5	5	+5	4	4	4	4	4	+4	3	0	0	0	0	—
3	1	1	1	1	1	—	0	1	0	0	0	—	2	2	2	2	2	—
4	1	1	0	0	0	—	3	2	3	3	3	+3	0	0	0	0	0	—
5	2	1	2	2	2	+2	0	0	0	0	0	—	4	4	4	5	3	+3
6	1	3	2	2	2	+2	0	0	0	0	0	—	0	0	0	0	0	—
7	1	1	1	1	1	—	6	6	3	3	2	—	0	0	0	0	0	—
8	4	4	4	4	4	+4	1	1	5	5	6	+6	0	0	0	0	0	—

Note on the experiment:

The colony formed in the laboratory is maintained almost as long a period as in natural habitat, and the limpets creep out of the home and feeds on brown sedentary microscopical organisms, mainly naviculoid forms, which cover the darker side of the glass wall, just as they do in nature (Fig. 8). The trace of cleanly eaten path made by the limpet is shown in Fig. 8.

In Fig. 8, one limpet, 22 mm. in shell length, is shown, which crept away from the home on its way of feeding. It was found on early morning of Oct. 31st and continued its feeding till 2 p.m. of the same day, and at 4 o'clock, the limpet returned to the home. At 9 o'clock on the next morning, this limpet was again found on the undermost end of the left path, and this figure was photographed at 6 o'clock on Nov. 1st.

Exp. 2. On the limpet after dispersion.

On Oct. 15th the limpets were found scattering here and there on the rock face at Mourajima. These scattered limpets were collected and were tested in the laboratory by the same method already described (Table VII).

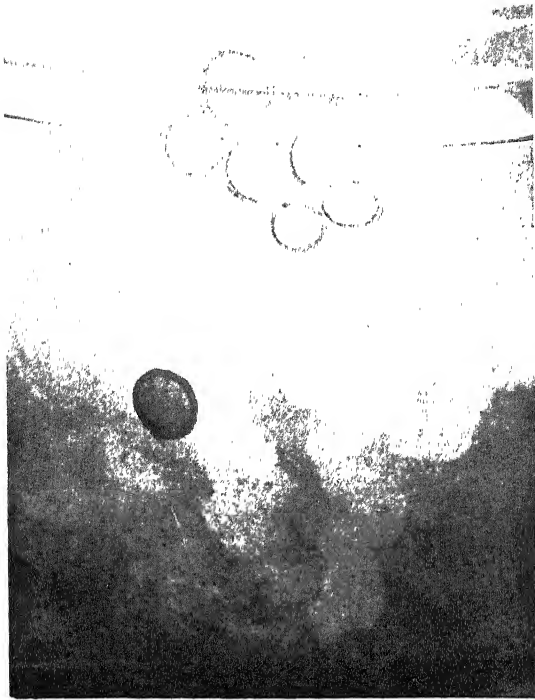


Fig. 8. A view of *Acmaea dorsuosa* showing the food-paths and colony formed in the glass vessel. Photographed on Nov. 1st, 1931.

TABLE VII.

Colony formation of the limpet after dispersion.

Exp.	No. 4. (Oct. 15th.)						No. 5. (Oct. 25th.)					
Temp.	19.0°C-10.0°C						18.9°C-9.0°C					
Days after												
Section	1	2	3	4	5	Colony	1	2	3	4	5	Colony
1	1	0	0	0	0	—	2	1	1	1	1	—
2	2	2	2	2	2	—	4	3	3	3	3	—
3	3	3	3	3	1	—	0	0	0	0	0	—
4	0	0	0	0	0	—	0	0	0	0	0	—
5	3	5	4	6	6	+5	2	5	7	7	9	+8
6	2	0	0	0	0	—	0	0	0	0	0	—
7	4	5	5	5	7	+7	6	4	2	2	0	—
8	1	1	2	0	0	—	0	0	0	0	0	—



As we can see in Table VII, the limpets which once dissolved a colony in their natural habitat when those were brought into the laboratory.

Exp. 3. On the young limpet.

On Dec. 5th I collected some limpets about 2 years of age, and examined them by the same method. They did not form a home within the length of time allowed for the formation of a home by old limpets, so I waited another ten days and found only one small home made by three limpets (Table VIII). It seems safe to conclude that young limpets, two years of age, normally do not form a colony.

TABLE VIII.

Colony formation of the young limpet of two years of age.

Exp.	No. 6. (Dec. 5th.) 9.0°C 3.0°C										Colony
Days after											
Section	1	2	3	4	5	6	7	8	9	10	
1	3	3	3	2	2	2	2	2	2	2	—
2	3	3	2	2	2	2	2	2	2	2	—
3	2	2	3	3	3	3	3	3	3	3	—
4	1	1	1	2	2	2	1	1	1	1	—
5	5	5	5	5	4	5	6	6	6	6	+3
6	0	0	0	0	0	0	0	0	0	0	—
7	1	1	1	0	1	0	0	0	0	0	—
8	1	1	1	1	1	1	1	1	1	1	—

Exp. 4. In the dark room.

Five young limpets and eleven older ones more than 4 years of age, were used in the experiment. The glass vessel was covered with a box. The experiment began on Dec. 16th and on 27th. The limpets formed two colonies. The dark box was then taken away and on Jan. 3rd two new additional colonies were found as shown in Table IX.

It is to be emphasized in the present experiment that the limpet can form a colony in the dark. Whether or not the limpets tend to form the colony in darker or lighter sides of vessel were tested and the results are shown in Fig. 9.

From Fig. 9, it can be said that though the majority of the limpets form colonies in the darkest place, but as in sections 5 and 6 in A and 7 in B, which receive the most light, a few colonies were formed there. So I think that the colony can be formed both in darker and lighter places in the laboratory.

TABLE IX.  
Colony formation in the dark room.

Exp.	No. 7. (Dec. 16th.)							No. 8. (Dec. 29th.)				
Temp.	10.3°C-3.8°C							5.9°C-1.8°C				
Days after												
Section	2	4	6	8	10	12	Colony	13	15	17	19	Colony
1	1	1	2	2	3	3	+3	3	3	2	3	+3
2	3	2	0	1	1	1	—	0	0	0	0	—
3	1	1	2	1	1	1	—	1	1	0	0	—
4	1	2	4	4	4	4	+2	3	3	2	2	+2
5	0	0	0	0	0	0	—	0	0	2	2	+2
6	1	1	0	0	0	0	—	0	0	0	0	—
7	3	3	2	3	3	3	—	4	4	5	1	—
8	1	1	1	0	0	0	—	0	0	0	3	+3

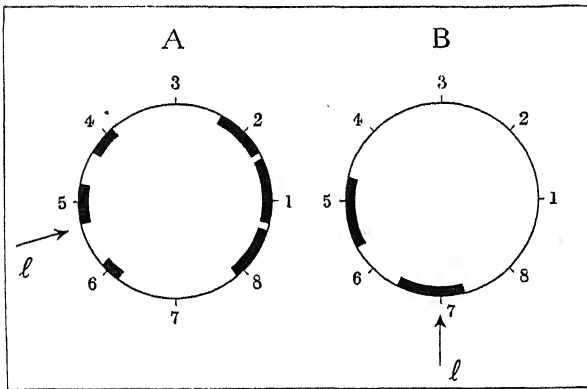


Fig. 9. Showing the positions where the colonies were formed in the glass vessel.  
A . Exp. 1. B....Exp. 2. *l* indicates the direction of light.

### c) General Consideration on the Experiment.

In Exp. 1 and in Exp. 2, it was shown that the limpets form colonies whether they were collected from home or whether they were collected after dispersion. It seems natural to assume that the limpets after once dissolving their home would not reform the colony so soon as was shown in Exp. 2, but contrary to an expectation the dispersed limpets reformed the home. In this connection one may doubt whether the limpets merely assembled on the darker side of the vessel by photic reaction and not by the impetus of colony formation habit. However we found that the limpets

collected after dissolution of their home reformed the colony in both darker and lighter positions as shown in Table IX and Fig. 9. Thus we can at once dispense of the idea that the limpet in question merely assembled and it was not the result of home making. Therefore, the instinct of colony formation may not be immediately extinguished after dispersion. It is also of interest to note that the limpets which formed the colony in the laboratory (Exp. 1) not only maintained it till Jan. 30th, but showed feeding habit as in the natural habitat. Thus the formation and dispersion of colony are possible under somewhat unnatural environments.

#### SUMMARY

1. *Acmaea dorsuosa* GOULD shows variation as to the time of dispersion, according to the age of the limpets, younger limpets less than three years of age disperse comparatively later than older ones of more than 1 years of age. And the dispersion begins from the colony over which waves splash more strongly.

2. The dispersion of the limpets seems to be related to the steady and continuous fall of atmospheric temperature below 15°C in its minimum temperature during late autumn season, and the reformation of the colony seems to begin during the spring season when the atmospheric temperature rises steadily and continuously till about 13°C (in its maximum temperature) or more.

3. The frequency of vertical distribution of the young limpet less than three years of age is almost symmetrical, and in the first three months after dispersion, the limit of range is nearly one meter above and below the home level, but in the later days, the position of maxima moves nearly 60 centimeters below the home level.

4. The frequency of vertical distribution of the older limpets more than 4 years of age is not symmetrical, and in the first three months after dispersion, the limit of range is two meters above and one meter below the home level, while in the later days the range changes from 40 centimeters above to about 160 centimeters below the home level.

5. On the horizontal dispersion, the maximum distances traversed to both direction are about the same, showing about six meters.

6. In the next spring, the colony of the limpets is formed newly with new members added, but one part of them returns exactly to their original home of the former year.

7. The scar of the limpet is marked on the rock within five months,

and it is not extinguished in a year, so there are seen double or even triple scars.

8. The formation of a colony is possible under somewhat unnatural environments. Since by the fact that the dispersed limpets reformed the colony and it follows that the nature of colony formation may not be immediately extinguished.

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# ON THE CYTOPLASMIC FRAMEWORK OF THE PLASMODIUM, *PHYSARUM POLYCEPHALUM*.

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(Received May 9, 1933)

Studies on the physical structure of cytoplasm have led to two views: one, that living matter is an emulsion without any other structural elements than those inherent in such a system. This is the opinion held by HEILBRUNN<sup>1)</sup> based on his results in viscosity measurements with the centrifuge method, and by CHAMBERS<sup>2)</sup> relying on his examination of protoplasm with the micro-needle. The other view is that the clear substance of the protoplasm has a fine permanent structure which resists deformation by the centrifuge. CONKLIN<sup>3)</sup> says: "On its morphological side the polar organization (of the egg) consists in a relatively persistent framework of viscid material which is also elastic and contractile so that it tends to resume its normal form when distorted." The recent studies of WINTREBERT<sup>4)</sup> on the rotation of the eggs of *Discoglossus* lead him to postulate a "cytosquelette" or a "trame resistant" to account for the persistence of form in the egg. NEEDHAM<sup>5)</sup> discusses the importance of the problem and the necessity for its solution. Among the botanists, SEIFRIZ<sup>6)</sup> and SCARTH<sup>7)</sup> both speak for an elastic structure in the protoplasm of plant cells, the latter thinking of such structure in terms of "brush heap grouping of chains of molecules."

Most of the evidence on the subject is from experiments with eggs. But there are two disadvantages in an attempt to draw general conclusions from a study of living matter in eggs for the reason that they contain a large amount of non-living material as inclusions, and furthermore the egg is a very special form of living matter, the end of which is to turn into another and totally different type of living thing. There is however a possibility of studying the structure of living matter in a stable adult form

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\*The author takes pleasure in thanking Dr. ELIZABETH BRADWAY for help with the experiments, and acknowledging a grant in aid from the Research Council of the University of Oregon.

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# ON THE RÔLE OF THE BRAIN AND CEPHALIC NERVES IN THE SWIMMING AND RIGHTING MOVEMENTS OF THE POLYCLAD WORM, *PLANOCERA RETICULATA*.

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(With 11 figures)

(Received June 30, 1933)

The strong, graceful swimming movements of the polyclads are a striking demonstration of a nice coördination which is apparently made possible by a nervous system of comparatively simple structure. Decapitation or removal of the brain, i.e. the central ganglion, results in immediate cessation of swimming movements and indeed of any spontaneous attempts at locomotion. This experiment was first performed by LOEB<sup>1)</sup> in his study of *Thysanozoon*. Similar effects were obtained by OLMSTED<sup>2)</sup> working with three California species of polyclads, and by myself<sup>3)</sup> with *Yungia aurantiaca* of Naples Bay. At first thought one might suppose the brain of the worm to be the center of spontaneity and coördination, and in fact the experiments of OLMSTED led him to suggest that this is true. But in *Yungia* I succeeded in proving that such is not the case, and that the function of the brain is rather to act as an amplifier of impulses, so that only a slight stimulation is needed to call the swimming mechanism completely into action. In fact it appears that in *Yungia*, even without the brain, the body possesses the complete neuromuscular mechanism for making coördinated swimming movements. Another way of viewing the matter is that the brain of the polyclad maintains a low threshold throughout the nervous system. When the brain is cut off the threshold of the nervous system is made high and consequently impulses of ordinary strength are not effective in causing swimming movements. HERRICK<sup>4)</sup> has recently used this concept to account for certain brain functions in mammals. If this view is correct it should be possible to elicit coördinated movements in the polyclad worms either by very strong stimulation or by lowering the threshold of the nervous system so that weak stimuli would become effective. As a matter of fact both methods have proven adequate, for

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I was able to show in *Yungia* that strong mechanical stimulation of the central nerve strands at the anterior end of a decapitated worm produced unmistakable swimming movements of the two lateral halves. Furthermore, spontaneous swimming through the water was shown by decapitated specimens when they were immersed in a sea water solution of phenol of dilution 1:40,000, the phenol in this case serving to reduce the threshold of the nervous system and also at the same time to provide excitation of the neuromotor apparatus. It was found in general that the monovalent cations Na and K, also the nerve excitants strychnine and nicotine conferred on the decapitated worms at least partial spontaneity for coördinated swimming movements. The experiments then proved that in *Yungia* the body apart from the brain contains the necessary mechanism for spontaneous coördinated movements; the brain affects the system by making it more delicately responsive.

Operative experiments on *Yungia* were never very satisfactory because

of the fragility of the animal, and it was therefore very gratifying to find at Misaki during the present summer adequate numbers of the large polyclad *Planocera reticulata*<sup>5)</sup>. This worm has a body of well knit, tough texture, well adapted to operative procedures. Moreover, with the aid of a pair of binoculars the brain and nerve plexuses are readily seen in the inverted animal. This clear visibility of the ventral nervous system makes it possible to observe very precisely the anatomical results of operations. The brain ap-

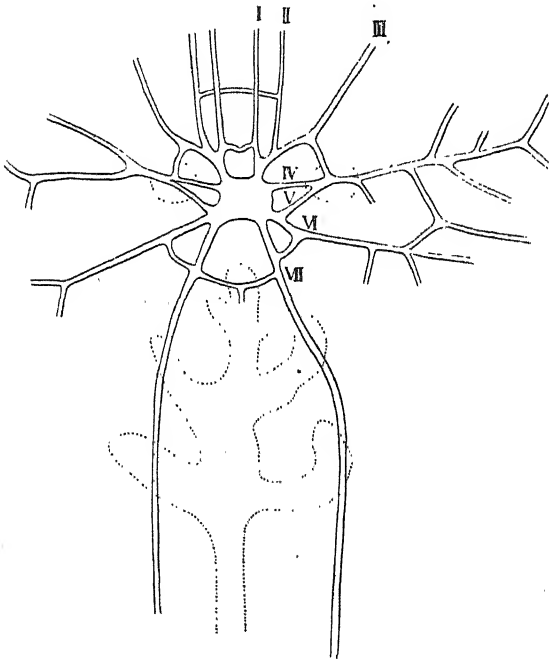


Fig. 1. Large scale view of the brain and cephalic nerves drawn from life. The Roman numbers for the main nerves as indicated on the right side are as used in text.

pears as a yellowish, rectangular body between the right and left dorsal tentacles. From it proceed on each side seven large nerves (Fig. 1). Peripherally these nerves with many anastomoses run to the margins of the animal, so that while the nerves radiate from the brain they they also form a network which ramifies throughout the ventral side of the animal (Fig. 2). Nothing of the dorsal network could be seen hence it is concluded that this part of the nervous system is structurally very fine.

As in most animals with a creeping habit the polyclad worm is provided with dorsal and ventral flexing musculature. This arrangement makes possible the swimming movements which consist in successive waves of dorsal curling of the lateral halves of the animal, each wave succeeded by a beat downward, i. e. ventralward. The waves proceed from head to tail. Thus in swimming the muscle groups used are the dorsoflexors and ventroflexors which are thrown into action metachronously. If now, an active animal is decapitated or the brain removed, swimming ceases and the body deprived of its brain remains on the bottom quiescent;

the head containing the brain swims about. Frequently it is possible in a decapitate preparation to elicit characteristic reflexes by means of gentle mechanical stimulation. Thus a touch with the forceps near the margin results in an upward curling of the edge for a few millimeters of length. If the locus of excitation be moved nearer the center of the body, progressively greater longitudinal stretches of the margin are involved in dorsoflexure (Fig. 3). A contralateral response as a rule occurs and is

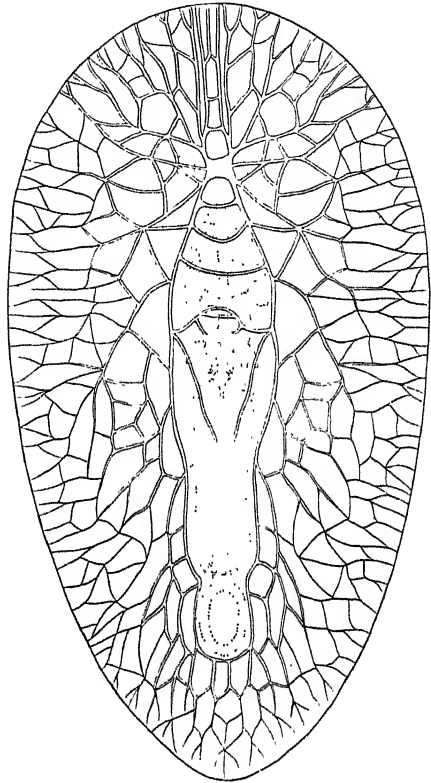


Fig. 2. Sketch of the entire nervous system as seen from the ventral side of a living specimen. Non-nervous structures dotted in. Semi-diagrammatic.

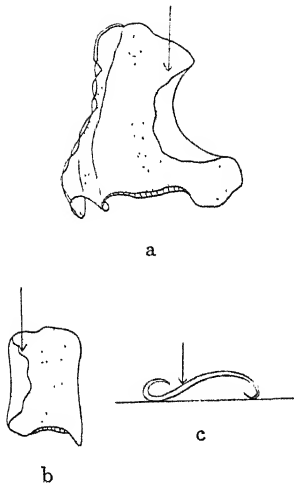


Fig. 3. Two decapitate preparations (a and b) responding to dorsal stimulation by pressure with forceps. Arrows indicate direction of stimulus which was applied at about the point of greatest curling. Cross section c showing the dorsal reflex for ipsilateral stimulation, ventral reflex for contralateral.

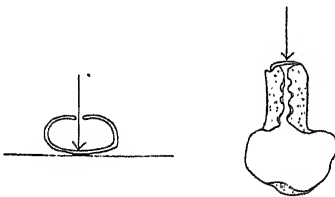


Fig. 4. Ventral reflex shown by a decapitate preparation receiving pressure stimulation in a median ventral locus.

more extensive if the point of excitation be near either end of the preparation. This response may take the form of either dorsoflexure or ventroflexure.

In a similar way ventral flexure of the margins may be demonstrated in an inverted animal by stimulation of the ventral surface (Fig. 4). That the periphery contains neuromuscular apparatus, adequate in itself for executing reflexes is shown by an experiment in which a longitudinal piece approximately 10 mm. wide was cut from the margin of a very large specimen. This piece responded to mechanical stimulation of the top surface by dorsal flexure of the outer edge. If put ventral side up on the bottom it righted itself; by dorsal reflex of a little of the margin some of the ventral surface is brought into contact with the bottom and it creeps actively along until the whole piece is dorsal side up. These results prove that the peripheral nervous reticulum contains all the elements essential to performing simple reflexes and that neither brain nor central nerve trunks are necessary.

Frequently decapitation results in complete ventral curling, the animal rolling up along the long axis, tail in, head outside. This evidently means that with the loss of the brain the tonus of the dorsal musculature is weakened, and that of the ventral musculature is increased and so predominates. Even in cases where the preparation flattens itself out on the bottom it may prove refractory and fail to respond to mechanical stimulation. However, if it be put into a sea water solution of phenol dilution 1:10,000, almost at once a heightened sensitivity becomes apparent: the margins begin slight, active, convulsive move-

ments, and in about three minutes the whole body flattens out and makes coördinated creeping and swimming movements. None of these efforts are successful in changing the animal's position, a result which is apparently due to the general action of the phenol, since in the control experiments in which whole animals were immersed in the phenol solution they became very active in making swimming movements without being able actually to accomplish locomotion. Thus the decapitate preparation and the whole animal behave exactly alike in a solution of phenol. Apparently the phenol renders the swimming stroke ineffective by reducing inhibitions to ventral bending, so that the tail makes backward pulling movements. The important point is that in animals without the brain, the nervous system has its sensitivity so heightened by phenol that there is a return

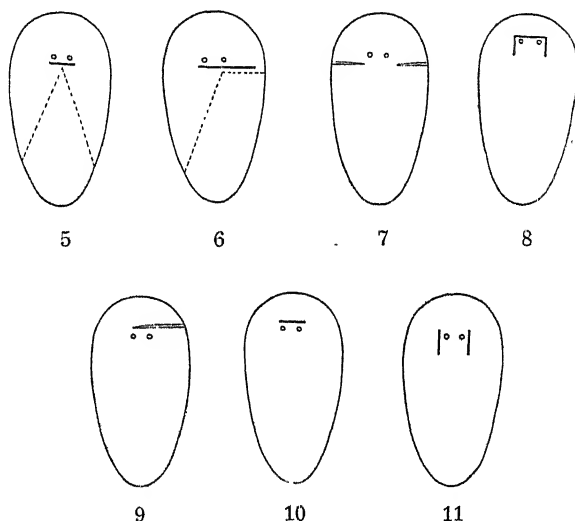


Fig. 5. Diagram showing effect of cutting nerve pair *vii*. The posterior sector enclosed by broken lines is inactive in swimming and assumes an arched posture due apparently to the dominating tonus of the ventral musculature.

Fig. 6. Same as fig. 5 except that the incision has been carried to the right leaving the margins attached by a narrow strip. The paralyzed area now involves the entire right side posterior to the incision. The marginal bridge is "unable to carry the swimming impulses from anterior to posterior.

Fig. 7. The body posterior to the brain level is isolated except for nerve pair *vii*, yet swimming is unimpaired while righting is interfered with.

Figs. 8-11 inclusive show incisions severing anterior and lateral cephalic nerves but in all cases leaving pair *vii* intact. As a result of these operations righting is impaired but the swimming rhythm of the body is normal.

of spontaneous movement which closely resembles the normal. This proves that the neuromuscular system without the brain is adequate to carry out the complicated movements of swimming provided the threshold is made sufficiently low, as it is in this case by phenol.

Further and detailed proof that in *Planocera reticulata* the brain functions in swimming by sending impulses directly to the musculature over paths which cover a limited area, is furnished by a series of operative experiments. 1) If the two central posterior nerves (*vii*) are severed just behind the brain, the worm continues to swim but a sector comprizing about one quarter of the body is immobilized (Fig. 5). This paralyzed part is not merely flaccid but takes an arched position, evidently the result of the predominant tonus of the ventral musculature. The swimming waves stop at the bounds of this sector. 2) If now the incision be carried to the right, severing in addition to nerves *vii*, also right *vi* and posterior branches of *v*, leaving the right side connected by a marginal strip about 5 mm. deep, then when the worm swims all the right half of the body posterior to the cut is inactive; the swimming rhythm is not carried by the nerve plexus of the marginal bridge (Fig. 6). These results then mean that in conducting impulses for swimming movements the more finely reticulated peripheral nervous system is not effective if its direct connection with the brain through nerves *vii* is impaired, a fact which may depend upon the rule that in a nervous system containing nerve cells the impulse diminishes in effectiveness as it proceeds from the point of origin<sup>9</sup>. The factor which seems to be the principal one in disturbing swimming reflexes when nerves *vii* are cut is the ventral curvature and immobility of the posterior part of the animal. It would seem then that in addition to carrying impulses for the swimming rhythm to this region, pair *vii* have an inhibitory action on the ventroflex musculature. 3) Confirmation of this view is given by a converse experiment in which the incisions were made on either side from nerves *vii* to the margins, thus leaving pair *vii* intact but severing everything else peripheral to them (Fig. 7). An animal prepared in this way swims normally with the posterior quarter completely relaxed as in the normal. Hence the inhibitory impulses to the ventroflex musculature of the posterior part pass by the *vii* pair alone. Likewise the metachronism of the lateral halves posterior to the level of the brain seems to be completely carried by the *vii* pair. On the other hand, the righting of such a preparation is accomplished only with difficulty. This means that righting movements are mediated by nerves anterior to pair *vii*. 4) If the brain be isolated

anteriorly and laterally by cutting the roots of pairs *i-vi*, i. e. all except the *vii*, (Fig. 8) then when inverted the animal makes efforts to right itself for some time both at head and tail ends, the two regions pulling against each other very much as the starfish does when the circumoral nerve ring is severed in one place<sup>6</sup>. This proves that in the normal animal the righting impulses entering the brain from the anterior pairs of nerves determine the course of the reaction, a part of this function being to set up inhibitory impulses from the brain to the parts of the body posterior to it.

In general it may be said that operations severing nerves are without effect on the swimming movements of the body posterior to the brain level provided pair *vii* is intact, but the cutting of anterior or lateral branches always impairs righting. For example fig. 9 shows an operation in which right nerves *i*, *ii*, and *iii* were severed. In this animal swimming was unimpaired but righting difficult. The same was true of the animal operated as shown in fig. 10, in which the nerve pairs *i*, *ii*, and *iii* were cut in an animal which before the operation gave especially lively and prompt righting. After the anterior nerves were severed righting was slow and difficult, head and tail sometimes pulling against each other. Similar results were obtained in an animal operated on as in fig. 11, in which nerve pairs *i*, *ii* and *vii* were left intact, the pairs *iii*, *iv* and *v* with branches of *vi* being severed. In this animal the righting movements were disorganized; often two parts at opposite sides of the animal taking hold of the bottom and pulling against each other. The swimming rhythm is perfect but the animal rolls unsteadily as if the balancing apparatus were not well adjusted; if the tail touches bottom it attempts to hold fast even when the worm is swimming.

In conclusion, the experiments show that in *Planocera reticulata* the brain functions as an amplifier of impulses in such a way as to maintain the neuromuscular mechanism in a state of delicate sensitivity. With the loss of the brain the threshold of reactions is raised throughout the system and the body is quiescent except when subjected to strong stimulation, or has its sensitivity restored by phenol. In the latter case the decapitated worm shows the same spontaneity and makes the same swimming movements as the normal animal in the same solution. There are seven pairs of cephalic nerves, of which the anterior and lateral ones, i. e. the first six, are essential to righting and possibly also to balancing in swimming. In righting, some of the impulses which reach the brain by these nerves are converted into inhibitions for the activity of the posterior half of the

body, i. e. in cases where the anterior or lateral cephalic nerves are severed, different parts of the body compete and retard righting. The posterior pair *vii* cephalic nerves are concerned with the swimming rhythm; if they are intact swimming is normal, if one is severed the corresponding posterior part of the body loses its power of making swimming movements. Since the swimming impulses originate at the anterior end they may reach pair *vii* either through the brain alone or through the nerve reticulum laterally. The nerve reticulum alone is adequate to mediate dorsal and ventral reflexes.

It is a very welcome privilege to express my thanks to Professor YATSU, to Mr. YOSII and to Mr. ERI for their fine hospitality which made the days at Misaki a never-to-be-forgotten pleasure. To Mr. ERI I am especially indebted for much help in getting specimens of *Planocera*, for bringing helpful morphological literature to my attention, and for generously giving me the use, for purposes of comparison, of his own beautiful unpublished drawings of polyclads.

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# ON FUNCTION AND CHEMICAL DIFFERENTIATION IN THE NERVOUS SYSTEM OF COELOPLANA BOCKII.

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Several years ago in a series of experiments with invertebrates I obtained evidence which pointed to the conclusion that *pari passu* with increasing phylogenetic complexity, the chemical complexity of the nervous system increases<sup>1)</sup>. This fact is shown in two ways, namely, 1) by increasing sensitivity of the neurones to chemical excitants, and 2) by an increase in the number of neurophil substances which produce excitation. Thus, for example, coelenterates are sensitive to atropine at a dilution of 1:2000; starfish to strychnine and atropine 1:10,000 and nicotine 1:50,000; cephalopods (squid) react to strychnine 1:100,000 and to nicotine 1:1,000,000. In this respect the cephalopods are much like the vertebrates. As to the second criterion, the coelenterates give the spasm reaction only with atropine, echinoderms with atropine, strychnine and nicotine; oligochaets with atropine, strychnine, picrotoxin, and camphor; polychaets in addition react to caffeine, nicotine and phenol; cephalopods are more delicately responsive to the same series. Likewise the mammalian cortex is stimulated by all these chemical excitants and in addition by creatine<sup>2)</sup>.

When the opportunity presented itself to study *Coeloplana* at the Misaki Station it seemed to me that it should be possible to throw some light on the question of the position of the nervous system of this form in the phylogenetic series, by means of the animal's reactions to the neurophil alkaloids. To those not familiar with coeloplana it may be well to note that in the larval, free swimming stage it is unquestionably a ctenophore, with eight rows of comb plates and two tentacles. In the adult stage the comb plates are lost, the tentacles retained; a distinct ventral side develops from a flattening out of the outer half of the larval pharynx. This ventral surface has a strong tendency to cling to surfaces

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and plays the chief part in the positive stereotropism of the animal. The aboral side contains at the center the "sense organ". The tentacles move about freely, showing even when detached, extension, and retraction on stimulation. Locomotion is accomplished by muscular movement or by ciliary action.

Now, coeloplana, lying as it does in classification between the ctenophores and flatworms possesses extraordinary interest to the student of the phylogenetic development of the nervous system. Histological studies by means of methylene blue *intra vitam* staining indicate a nerve net structure extending throughout the body<sup>9</sup>. Morphological studies then suggest a similarity if not identity in type between the coelenterate and coeloplana nervous systems. The chief point in this is that there are no notable concentrations of nervous tissue in strands and ganglionic masses as in the echinoderms and worms, although ABBOTT<sup>11</sup> supposes there may be ganglia about the base of the aboral sense organ.

In preparing the specimens of coeloplana for the experiments the individuals were gently removed by means of a pipette from the alcyonarian on which they live, and put into glass dishes containing clear filtered sea water. The little animals soon spread themselves out on the bottom of the dish; a few extend themselves ventral side up against the surface film of the water. These last, since they remain for long intervals practically motionless, give an excellent opportunity for observing with the binoculars something of the animal's internal anatomy. At a room temperature of 20°-25°C. and in shallow layers of water, coeloplana may remain in good condition in the laboratory for several days. Low temperatures, however, are deadly and if put into the ice box (5°-10°C.) the little animals quickly die.

If now we proceed to study the reactions of a specimen of coeloplana which is extended and attached to the bottom of the dish, we shall find that the first response to mechanical stimulation of the margin is retraction of the tentacles. This proves that there is nervous connection between the retractor muscles and the sensory epithelium of the margin. Next the body tissue slowly withdraws from the locus of excitation, moving centralward; the excitation causes contralateral extension. The total effect of both reactions is to move the animal away from the point of stimulation.

Coeloplana rights itself when put dorsal side down on the floor of the dish. At first the margins are drawn together ventrally so that the animal forms an irregular ball-shaped mass; then muscular movement causes it to tumble about a bit. Eventually the ventral surface of an

edge touches bottom and it begins creeping away from the mass. Successively more and more of the ventral surface comes in contact with the bottom and attaches so that eventually the entire animal is spread out. It requires about 15 minutes for extension to be complete, and the animal comes to rest. This observation shows that the ventral surface of coeloplana is positively stereotropic, and that contact on the dorsal side has a kinetic effect causing continuous slow muscular movements. The situation is dynamically similar to that in some of the starfish<sup>7</sup>.

If we now proceed to test the sensitivity of coeloplana to chemical excitants, we shall find them always reactive to strychnine, atropine, nicotine and phenol. If a specimen is exposed to a solution of strychnine sulphate 1:5,000, spasmodic twitchings of the margin begin in about a minute and a half. Mechanical stimulation applied just inside the margin now causes dorsal flexure, and a contralateral reaction also of dorsal flexure, with subsequent movements toward the center of the animal, i. e. the net effect of the response to mechanical stimulation is to crowd the animal into a ball, by all of the tissues moving centralward. This is an entirely different picture from the one we have seen in the normal in which the animal moves *away* from the point stimulated. The behavior of the strychninized animal may then be regarded as a case of reversal of normal response. In cases of thorough strychninization of coeloplana I have seen opisthotonus of the whole animal, when in response to stimulation both tentacular ends bent dorsally in spasm. Strychninized animals always give up their hold on the bottom and lie knotted and twitching — showing primitive but unmistakable strychnine spasms.

Similar excitatory effects were obtained with atropine sulphate in sea water solution 1:10,000, with nicotine 1:10,000, and with phenol 1:10,000, a series very similar to that for echinoderms and worms. When we contrast this with the reactions of the sea anemone *Anthopleura*, which shows no effect of strychnine even when the crystals are put on the disk, and gives only sluggish responses under atropine 1:2,000 and nicotine 1:2,000, it is apparent that the nerve substance of coeloplana is nearer to that of echinoderms and worms than it is to that of the coelenterates. This fact constitutes an interesting problem. If the nervous system of coeloplana is chemically at a stage of development comparable to that of the worms then why should it be so much less effective? The obvious difference lies in the fact that the nervous system of coeloplana is structurally disposed like that of the coelenterates, and shows no morphological resemblance to the highly developed arrangement in *Planocera*<sup>6</sup>. An in-

teresting if somewhat puzzling corollary to these facts regarding coeloplana is that morphological complexity does not go hand in hand with chemical differentiation.

It gives me pleasure to express thanks to Mr. ERI and Mr. YOSH of the Misaki Station for much help in securing specimens and materials.

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# STUDIEN ÜBER DIE BILDUNG ORGANISCHER SÄUREN IN GRÜNEN PFLANZEN.

## II. DAS VERHÄLTNISS ZWISCHEN DEM STICKSTOFF- UND DEM SÄURESTOFFWECHSEL IM GANZEN KÖRPER VON *BEGONIA EVANSIANA* ANDR.

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(Mit 13 Textfiguren)

(Eingegangen am 30. Juni 1933)

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### I. EINLEITUNG.

Die Eiweissstoffe sind im Pflanzenorganismus wie im Tierorganismus unaufhörlichen Umwandlungen, d. h. dem Abbau und dem Aufbau, unterworfen. Im Tierorganismus werden die Eiweissstoffe und andere stickstoff-

haltige Substanzen teilweise in der Form einfacher Endprodukte des Stoffwechsels täglich aus dem Körper ausgeschieden. Dagegen geht in höheren grünen Pflanzen im allgemeinen keine Spur stickstoffhaltiger Substanzen verloren, obwohl unlängst KLEIN und STEINER berichtet haben, dass Ammoniak aus allen Pflanzenorganen, insbesondere aus Blättern und Blüten, entweicht; das kann chemisch nicht festgestellt werden, weil es durch Exhalation verloren geht.

Der Stickstoff-Stoffwechsel im Pflanzenkörper ist deshalb ein sehr interessantes Problem in der Pflanzenphysiologie. Nachdem neuerdings RUHLAND und WETZEL gezeigt haben, dass bei gewissen Pflanzen, den sogenannten „Ammon-“ oder „Säurepflanzen“, zwischen dem Stickstoff- und dem Säurestoffwechsel enge Beziehungen bestehen, wurde der Frage nach dem Verlauf des Stickstoffumsatzes bei grünen Pflanzen wieder wachsende Aufmerksamkeit geschenkt. Nach ihrer Ansicht entgiften die organischen Säuren, die sehr wahrscheinlich aus den durch Desaminierung entstandenen Kohlenstoffgerüsten hervorgehen, in gewissen Säurepflanzen das Ammoniak, ganz wie das nach EHRLICH, KOSTYTSCHEW und anderen in den Pilzen der Fall ist. Bei vielen anderen Pflanzen, den „Amidpflanzen“, wird das Ammoniak in Form von Asparagin gebunden und dadurch entgiftet und reserviert.

Der Zweck dieser Untersuchungen besteht darin, mit der quantitativen mikrochemischen Methode diese Frage zu klären, und zwar das Verhältnis zwischen dem Stickstoff- und dem Säurestoffwechsel im ganzen Körper von *Begonia Evansiana* ANDR.

## II. MATERIAL UND ARBEITSMETHODE.

Als Versuchspflanze diente mir auch hier wie in meiner ersten Mitteilung *Begonia Evansiana* ANDR., die aus am 20. Okt. 1932 gesammelten Bulbillen nach derselben Kulturmethode wie früher gezogen wurde. Zur Anwendung kam stets frisches Material, da erstens das Trockengewicht der Pflanze Tag und Nacht beträchtlichen Schwankungen unterliegt, weil zweitens der Eiweisstoff vom löslichen Stickstoff vollständig getrennt werden kann, und weil drittens nach SMIRNOW, IWANOFF und anderen Autoren beim Trocknen des Frischmaterials ein grosser Verlust an präformiertem Ammoniak zu befürchten ist und weiter andere Umsetzungen unkontrollierbar vor sich gehen.

Zur Bestimmung sowohl des Säuregehalts als auch der Stickstoffmenge in ein und demselben einzelnen Pflanzenteile wurde die Mikromethode verwendet.

Geringsten Mengen des Untersuchungsmaterials, Schnelligkeit der Arbeit, Billigkeit und Sauberkeit, die die Apparatur gewährleistet, sind die wesentlichen Vorteile dieser Mikromethode. Es wurden im allgemeinen folgende sechs Stickstofffraktionen experimentell oder durch Rechnung quantitativ ermittelt: 1. Gesamt-Stickstoff, 2. Eiweiss-Stickstoff, 3. gesamter löslicher Stickstoff, 4. Ammon-Stickstoff, 5. Amid-Stickstoff und 6. Amino-Stickstoff.

### A. Stickstoffbestimmung.

#### 1. *Bestimmung des Gesamt-Stickstoffs.*

Zur Bestimmung der N-Fractionen 1–3 bediente ich mich der von F. PREGL eingehend beschriebenen Mikro-KJELDAHL-Methode. Das stickstoffhaltige Untersuchungsmaterial, dessen Zubereitung unten noch näher beschrieben werden wird, wurde im gewöhnlichen langhalsigen KJELDAHL-Kölbchen (15 ccm Inhalt), unter Zusatz einer Messerspitzevoll eines vorrätig gehaltenen Gemisches von einem Teil Kaliumsulfat und drei Teilen Kupfersulfat als Katalysator, durch 2 ccm konzentrierter Schwefelsäure (Sp. Gew. 1.84 „zur Analyse“ von KAHLBAUM) zersetzt und ausserdem zur Vermeidung des Stossens oder heftiger Aufwallung infolge Siedverzögerung, in je ein Kölbchen ein Glaskügelchen von etwa 1/20 ccm Volum getan und auf ein Zersetzungsgestell gebracht. Um grosse Verbrennungsreihen gleichzeitig oder nacheinander durchführen zu können, verwendete ich dabei ein Glasgestell, welches seitlich mit sechs Ansätzen versehen war; damit war zugleich Sauberkeit und Abzug der Dämpfe ermöglicht. Früher verwandten mehrere Autoren Glasrohre mit grossem Durchmesser als Verbrennungsapparat, um die bei der Veraschung der organischen Substanz frei werdenden Dämpfe darin aufzufangen und mittels der Wasserstrahlpumpe abzusaugen. Damit ist dieser Zweck ausgezeichnet erfüllt worden. Andererseits ist aber ein neuer Fehler hinzugekommen, der noch stärker als bei den Versuchen unter dem Abzug zutage tritt, denn weil die durch die Wasserstrahlpumpe in das Glasrohr gesaugte Luft der Laboratoriumsatmosphäre mehr oder weniger Ammoniak enthalten kann, so muss zunächst das in der durch die Wasserstrahlpumpe aufgesaugten Luft enthaltene Ammoniak vor dem Durchströmen in den Verbrennungsapparat beseitigt werden. Aus diesem Grunde verfuhr ich, wie folgt: Die Luft wurde zuerst durch ein 16 cm langes, mit Baumwolle gefülltes Glasrohr von 2 cm Weite und dann durch eine spezielle, mit konzentrierter Schwefelsäure gefüllte Gaswaschflasche durchgeleitet, um dadurch  $\text{NH}_3$ -

freie Luft mit beinahe konstanter Stromgeschwindigkeit durch das Verbrennungssammelrohr treiben zu können. Die Gaswaschflasche ist so hergestellt, dass der Luftstrom in recht feine Bläschen zerteilt wird und dabei alle Luftteilchen mit der Schwefelsäure in Berührung kommen. Die dadurch ammoniakfrei gemachte Luft streicht nun an allen seitlichen Ansätzen vorbei, bevor sie am anderen Ende des Verbrennungsrohrs wieder austritt und in das Rückschlagsventil mündet, indem sie den grössten Teil des bei der Verbrennung des Materials zuerst abgehenden Wasserdampfs niederschlägt und später der grösste Teil der sauren, bei der Aufschliessung der organischen Substanz entwickelten Dämpfe zurückgehalten wird. Das Verbrennungsrohr besteht aus einem Glasrohr von 4 cm Durchmesser, an das sechs kurze Rohrstücke, die alle erst schräg nach oben gerichtet und dann in einem Winkel von 90° nach unten gebogen sind, angeschmolzen sind. Das Verbrennungskölbchen ist an das Verbrennungsrohr mit dem Gummistück gasdicht angeschlossen. Der Kölbchenboden ruht auf einem Stahldrahtnetz, unter sich sechs Mikrobrenner befinden. Diese Anordnung gewährleistet ein rasches, gleichmässiges Erhitzen des Kölbchens und vermeidet die Gefahr eines Risses, mit der sonst bei direktem Erhitzen des am Ende der Verbrennung nur sehr wenig Flüssigkeit enthaltenden Kölbchens in hohem Masse zu rechnen ist. Innerhalb sehr kurzer Zeit tritt Verkohlung ein und bald darauf eine ziemlich starke Entfärbung der Flüssigkeit (etwa nach 15 Minuten). Zur Beschleunigung der weiteren Verbrennung ist hier, anstatt reinsten Perhydrols, reinste Überchlorsäure (Sp. Gew. 1.20, etwa, 30% „zur Analyse“ von KAHLBAUM) verwandt worden, indem man 2–3 Tropfen der noch heissen Flüssigkeit vorsichtig zusetzte. Die Zersetzung wird durch weitere Erhitzung (etwa 2 Minuten) zu Ende geführt. Wenn das Untersuchungsmaterial keine grössere Menge von Nitraten enthält, kann man einfach den Gesamtstickstoff in Form schwefelsauren Ammonium wieder finden. Nach vorheriger Verdünnung mit 2 ccm Wasser wird das Ammoniak durch Zusatz von 15 ccm 30%iger Natronlauge, die überdies 5% Natriumthiosulfat enthält, überdestilliert und in Salzsäure aufgefangen. Das Kühlrohr war aus Silber gefertigt und hat sich vor allem wegen der leichten Kühlung, der Unzerbrechlichkeit und auch des relativ niedrigen Preises an Stelle des Kühlers aus Quarz gut bewährt. Zum Auffangen des überdestillierten Ammoniak in Salzsäure diente ein 100 ccm Inhalt fassendes Kölbchen aus PYREX-Glass, das vorher gut durchgedampft worden war. Als Titrierflüssigkeit dienten je n/100 Salzsäure und Natronlauge, denen, um den Vergleich der umgeschlagenen Farbe der Flüssigkeit mit der der typisch

basischen oder sauren zu erleichtern, bereits eine bestimmte Menge Indikatorsubstanz zugesetzt war. Als Indikator wurde Methylrot verwendet. Der Umschlag von rot zu kanariengelb kann nach einiger Übung hinreichend scharf erkannt werden. Bei Berücksichtigung aller obiger Umstände kann man mit einer Genauigkeit von 0.01 ccm n/100 der Lösung rechnen.

Auf diese Weise erhielt ich die Wert für den Eiweiss- und den löslichen Stickstoff, deren Summe den Gesamt-Stickstoff ergeben muss; in einigen Fällen wurde die Menge des letzten nach der KJELDAHL-Bestimmung an frischem Material weiter direkt als solcher ermittelt. Bei der Angabe des Gesamt-Stickstoffs ist der Nitrat-Stickstoff ausgenommen.

### 2. *Bestimmung des Eiweiss-Stickstoffs.*

Das Material wurde mit einem sauberen Schreibpinsel von Staub säubert, mit einem scharfen Messer zerschnitten und in einem Wägegläschen ganz genau gewogen. Dann wurde es unter Zusatz einiger Tropfen reinsten Toluols im Mörser möglichst fein zerrieben, sodass fast alle Zellen geöffnet wurden. Als Fällungsreagens der Proteinsubstanz dienten mir, wie ENGEL das schon empfohlen hat, 3–5 ccm heisser 1%iger Tanninlösung, der 0.1%ige Schwefelsäure beigegeben war. Das Gemisch blieb etwa 1 Stunde im Mörser; dann wurde durch ein möglichst kleines, N-freies, quantitatives Filter filtriert. Der eiweisshaltige Rückstand wurde mit destilliertem Wasser mehrmals ausgewaschen und dann gelinde ausgepresst. Filtriert wurde er in ein 15 ccm Messröhrchen. Der Filtrerrückstand samt dem Filtrierpapier wurde nun nach KJELDAHL bestimmt. Die Benutzung eines möglichst kleinen Filters war notwendig, um den Abschluss nicht unnötig zu verzögern. Ich verwendete den 3½ cm-Filter von SCHLEICHER und SCHÜLL. Sowohl in konzentrierter Schwefelsäure als auch im Filter erwies sich jedoch die Menge des gebundenen Stickstoffs messbar. Die Analyseergebnisse mussten dementsprechend korrigiert werden.

### 3. *Bestimmung des löslichen Stickstoffs.*

Das Filtrat aus niedergeschlagenen Eiweisstoffen wurde bis zur Mark (15 ccm) des Messröhrchens aufgefüllt. 5 ccm dieser Lösung wurde ins KJELDAHLkölbchen getan und wie bei der Bestimmung des Gesamt-Stickstoffs weiter verarbeitet.



#### 4. Bestimmung des Ammon-Stickstoffs.

Zur quantitativen Bestimmung wurde zunächst das Ammoniak in der Restflüssigkeit des Filtrats, wie ENGEL empfohlen hat, im CLAISENkölbchen (etwa 50 ccm Inhalt), welches zwei kugelige Erweiterungen mit eingeschmolzenen Tropfenfängen hat, unter vermindertem Druck überdestilliert; und zwar ist der Analysenvorgang, wie folgt: 10 ccm des Eiweissfiltrats wurden in das CLAISENsche Destillationskölbchen pipetiert und dieses Kölbchen mit dem Kühler verbunden. 10 ccm Kalkwasser, das kein festes Calciumoxyd enthielt, genügten meistens, das Filtrat alkalisch zu machen. Aber wegen der stark sauren Reaktion des Filtrats von *Begonia* benutzte ich davon oftmals 15 ccm. Als Vorlage diente mir eine 30 ccm fassende Saugflasche aus hartem Glas, die mit 10 ccm n/100 Salzsäure beschickt war. Um das Ammoniak der Luft zu beseitigen, wurde die durch die Kapillare in das CLAISENkölbchen eintretende Luft vorher durch eine Gaswaschflasche mit konzentrierter Schwefelsäure geleitet. Das Destillationskölbchen tauchte in ein gläsernes Wasserbad, das auf 50°C erwärmt wurde. Wegen der Durchsichtigkeit des gläsernen Wasserbades ist es ratsam, das Erhitzen des Destillationskölbchens, statt im gewöhnlichen Wasserbade, in jenem vorzunehmen.

Es wurde vorsichtig evakuiert, bis lebhaftes Sieden einsetzte. Der Druck betrug 30–35 mm, die Siedetemperatur 30–35°C. Nachdem etwa 2/3 der Flüssigkeit überdestilliert waren, was ungefähr 10–15 Minuten in Anspruch nahm, endete die Destillation stets. Die das überdestillierte Ammoniak auffangende Salzsäure wurde in üblicher Weise mit n/100 Natronlauge titriert.

#### 5. Bestimmung des Amid-Stickstoffs (Mikro-SACHSSE).

H. ENGEL gelang es, in ein und derselben Lösungsmenge den Ammon- als auch den Amid-Stickstoff zu bestimmen. Die Prüfung nach seiner Methode brachte ganz befriedigende Ergebnisse, wie aus Tabelle 1 zu entnehmen ist.

TABELLE 1.

Nr.	Gemisch von	Gewicht jedes Bestandteils des Gemisches.(mg)	Berechneter Stickstoff. mg	Gefundener Stickstoff. mg	Prozent
1	Ammonsulfat u. Asparagin.	4.408	0.934(4)	0.931(0)	99.64
		2.487	0.527(4)	0.525(0)	99.54
2	Ammonsulfat u. Asparagin.	3.133	0.659(9)	0.659(4)	99.92
		1.807	0.383(0)	0.380(8)	99.43
3	Ammonsulfat u. Asparagin.	4.079	0.864(7)	0.861(0)	99.57
		5.143	1.090(8)	1.078(0)	98.82

Deshalb verfuhr ich in der Bestimmung des Amid-Stickstoffs folgendermassen: Der im Destillationskölbchen verbliebene Rückstand, der etwa  $\frac{1}{3}$  des zur Ammon-Stickstoffbestimmung benützten Eiweissfiltrats entsprach, wurde nach Zusatz eines kleinen Bimssteinstückchens meistens mit 1 ccm konzentrierter Schwefelsäure angesäuert. Dem Hals des Destillationskölbchens wurde ein Rückflusskühler aufgesetzt, und das seitliche Rohr war als Verschluss mit einem Quetschhahn versehen.

Nach  $2\frac{1}{2}$  stündigem lebhaftem Sieden auf dem Drahtnetz wurde die Hydrolyse der Amide für beendet angesehen. Die Hauptsäuremenge wurde mit 30%iger Natronlauge neutralisiert und mit Magnesiumoxyd endgültig alkalisiert. Der weitere Gang der Bestimmung war derselbe wie beim Ammon-Stickstoff. Die im Pflanzenreich bisher gefundenen Amide, Asparagin und Glutamin usw., wiesen ausser der an Carboxyl gebundenen  $\text{NH}_2$ -Gruppe noch eine Amino-Gruppe auf, die bei der Hydrolyse nicht abgespalten wird. Für die Berechnung des Amid-Stickstoffs wurde die gefundene N-Menge verdoppelt, um den Stickstoffgehalt des Amidmoleküls auszudrücken.

#### 6. Bestimmung des $\alpha$ -Amino-Stickstoffs (Rest-Stickstoff).

Der  $\alpha$ -Aminostickstoff wurde quantitativ einfach aus der Rechnung ermittelt, indem die Summe der Ammon- und der Amidfraktionen von der Gesamtmenge des löslichen Stickstoffs abgezogen wurde; denn wie MOTHES schon durch direkte Analyse des „Reststickstoffs“ fand und wie auch aus Versuchen von ENGEL hervorging, besteht dieser Teil der löslichen Fraktion fast nur aus  $\alpha$ -Aminosäuren.

#### B. Bestimmung der Oxalsäure in freier und gebundener Form.

Zu diesem Zweck wurden dieselben Methoden angewandt, die sich in meiner vorigen Untersuchung als zuverlässig erwiesen hatten. Früher wog ich das Untersuchungsmaterial direkt auf dem Platin- oder Kupferschälchen; diesmal wurde es zur möglichsten Vermeidung von Wasserverlust aus den Schnittflächen im Wägegläschen gewogen, dann auf das Schälchen mit einer feinen Pinzette getan, und schliesslich wurde wie vorher verfahren.

### III. VERSUCHSERGEBNISSE.

#### A. Tagesschwankungen des Säuregehalts in den Blättern.

ULLRICH untersuchte die Tagesschwankungen des Säuregehalts von

*Anemone nemorosa*, *Rubus idaeus*, *Begonia semperflorens*, *Lactuca sativa* und *Lactuca virosa* und schloss aus dem Säuregehalt im Verhältnis zum Trockengewicht, dass die Oxalsäure in den Blattflächen von *Lactuca virosa* und *L. sativa* nur ganz geringen Schwankungen unterliegt, da der Gehalt nach dem Morgen nur ganz wenig ansteigt.

Nach RUHLAND und WETZEL vermehrt sich freie Oxalsäure im allgemeinen in den Blättern von *Begonia semperflorens* vom Abend bis zum Morgen, und zwar so, dass niedrige Temperatur einen grösseren Säureanstieg zulässt als hohe Temperatur und dass sich bei jungen Blättern die freie Oxalsäure stärker vermehrt als bei alten.

Nach BENDRAT treten bei *Sempervivum glaucum* auch ausgesprochene Tagesschwankungen auf. Ihre Stärke hängt vom Entwicklungszustand der Organe ab. Bei alten und mittelalten Blättern nimmt die Säure über Nacht zu, bei jungen Blättern geht der Säuregehalt entweder zurück oder bleibt konstant. Unter den anderen untersuchten Blattsukkulenten stieg der Säurespiegel bei *Bryophyllum calycinum*, *Epidendrum ciliare* und *Vanilla planifolia* ebenfalls über Nacht an, während er bei *Mesembrianthemum cordifolium* unter den gleichen äusseren Bedingungen sank.

Vor kurzem studierte SCHWARZE den Säurestoffwechsel nichtsukkulenter Pflanzen und zeigte, dass bei *Nicotiana Tabacum* die Säure unter natürlichen Verhältnissen nachts zunahm und am Tage der Säurespiegel zurückging. Bei *Oxalis Deppei* verhielt er sich insofern grundsätzlich anders, als sie nachts absäuert, und zwar in hohem Masse unabhängig von äusseren Verhältnissen.

Um die Tagesschwankungen des Säuregehalts in der Blattspreite zu bestimmen, führte ich Analysen von Blättern der *Begonia* (Nr. 52), die 81 Tage lang gezüchtet worden war, aus. Wegen der asymmetrischen Ausbildung der *Begonia*-Blätter konnten wir sie nicht dem Mittelnerven entlang in zwei gleiche Teilen teilen, wie das bei der physiologischen Untersuchung sonst üblich ist. Deshalb teilte ich die Blätter quer durch, wie in Fig. 1 angedeutet ist. Die Blatthälfte für die Säurebestimmung am Tage wurde um 12–13 Uhr und die für die in der Nacht um 18–19 Uhr abgenommen und jedesmal sofort verarbeitet. Der Sonnenaufgang dieses Tages war um 5 Uhr 46 Minuten und der Sonnenuntergang um 16 Uhr 53 Minuten. Die Ergebnisse sind in Tabelle 2 zusammengestellt.

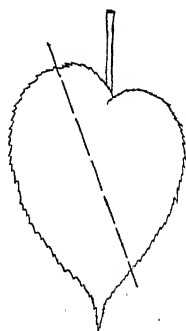


Fig. 1.

TABELLE 2.

Tagesschwankungen des Säuregehalts in der Blattspreite von  
*Begonia Evansiana* ANDR. Nr. 52. 81tägige  
 Züchtung im Gewächshaus.

Pflanzenteil	Frischgewicht d. Materials. mg	Oxalsäure mg	Prozent	mg Oxalsäure in 1000 g Frischgewicht.	Bemerkung
1. Blattspreite	275.297	2.346	0.85	8521.7	Tagesbestimmung
2. „	348.586	2.719	0.78	7800.0	
3. „	259.155	1.916	0.73	7393.2	
4. „	85.717	0.651	0.75	7594.7	
1. „	367.933	3.239	0.88	8803.1	Nachtbestimmung
2. „	409.522	3.379	0.82	8251.0	
3. „	355.980	2.918	0.81	8197.0	
4. „	129.398	1.023	0.79	7905.8	

Fig. 2 gibt obige Befunde kurvenmässig wieder. Aus der Tabelle und Figur kann man ersehen, dass der Säuregehalt in ganz jungen Blattspreiten geringer als in mässig gewachsenen ist und dass sich allgemein

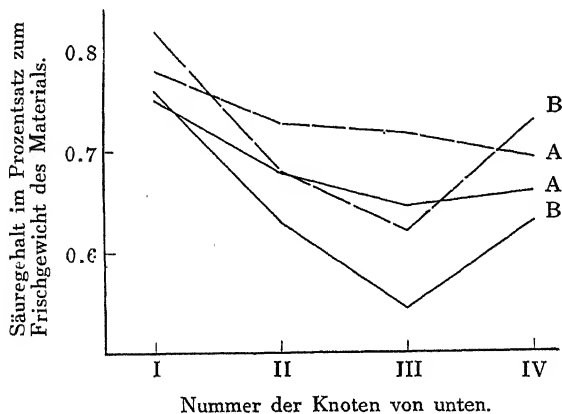


Fig. 2. Tagesschwankungen des Säuregehalts in der Blattspreite von *Begonia Evansiana* ANDR. A: Nr. 52; B: Nr. 55. 81tägige Züchtung im Gewächshaus.

— — — — — Nachtwerte      ————— Tageswerte

eine Tendenz zur Abnahme des Säuregehalts in der Reihenfolge der Knoten von der Basis nach der Spitze wahrnehmen lässt und in allen Blattspreiten die Säure über Nacht zunimmt. Der Säuregehalt dieses

Individuums erwies sich im Vergleich mit dem des vorjährigen als ziemlich grösser.

Die niedrige Temperatur während der Kulturzeit kann daran Schuld sein, da diese Versuchspflanze leider aus ganz äusseren Gründen zu spät in Wasserkultur gesetzt wurde. Es sei noch bemerkt, dass die Blattzahl bei diesem kleiner als beim vorjährigen Individuum war und die Blattspreite des jüngsten Blatts in den Bulbillen verändert war. Deshalb kann man nicht sagen, dass dieses Individuum sich normal entwickelt hatte.

Weiter gebe ich hier die Ergebnisse der Analyse bei einem anderen Individuum (Nr. 55) wieder, das auch 81 Tage in Kultur gewesen war. Die Blatthälfte zur Säurebestimmung am Tage wurde um 13–14 Uhr und die für die in der Nacht um 19–20 Uhr gewogen. Die Ergebnisse sind ähnlich wie die beim vorigen Individuum (Nr. 52) ausgefallen. (Tabelle 3 u. Fig. 2). Die Säurezunahme in der Nacht gegenüber der am Tage

TABELLE 3.

Tagesschwankungen des Säuregehalts in der Blattspreite von  
*Begonia Evansiana* ANDR. Nr. 55. 81tägige  
Züchtung im Gewächshaus.

Pflanzenteil	Frischgewicht d. Materials mg	Oxalsäure mg	Prozent	mg Oxalsäure in 1000 g Frischgewicht	Bemerkung
1. Blattspreite	282.586	2.430	0.85	8599.1	Tagesbestimmung
2. „	365.556	2.679	0.73	7328.5	
3. „	373.262	2.379	0.63	6373.5	
4. „	240.509	1.754	0.72	7292.8	
1. „	229.608	2.112	0.91	9198.2	Nachtbestimmung
2. „	359.933	2.823	0.78	7843.1	
3. „	313.156	2.256	0.72	7204.0	
4. „	235.071	1.954	0.83	8312.3	

ist bei den unteren, älteren Blättern geringer als bei den oberen, jüngeren, obwohl diese Neigung auch beim obigen Individuum wahrzunehmen war.

#### B. N-Stoffwechsel in den Bulbillen und Knollen.

E. SCHULZE fand, dass die unterirdischen Reservestoffbehälter einen sehr ansehnlichen Teil ihres Stickstoffs als lösliche Verbindungen aufspeichern. Neuerdings wiesen RUHLAND und WETZEL nach, dass in dem

Rhizom von *Rheum hybridum hort.* etwa zwei Drittel des Gesamt-Stickstoffs als löslicher Stickstoff und ein Drittel als Eiweiss-Stickstoff vorhanden ist. Von dem löslichen Stickstoff lässt sich nur wenig Ammoniak nachweisen. An einer grossen Zahl unterirdischer Reservestoffbehälter untersuchte GRÜNTUCH vor allem den N-Stoffwechsel der Kartoffelknollen im Verlaufe ihrer Vegetation und kam dabei zum Schluss, dass bei Kartoffelknollen der lösliche Stickstoff den Hauptteil des Gesamt-Stickstoffs darstellt und dass der Amid-Stickstoff beim N-Stoffwechsel des obigen Organs scheinbar eine bedeutende Rolle spielt.

Ganz kürzlich studierte RAHN mit seiner „Rohfaser-Methode“ den N-Stoffwechsel der vegetabilischen Speicherorgane von *Allium Cepa*, *Oxalis Deppei* und *Asparagus officinalis* und stellte dabei fest, dass Säurepflanzen (*Oxalis Deppei*) vor dem Auskeimen einen hohen Gehalt an Aminosäure aufweisen, in Amidpflanzen (*Allium Cepa*, *Asparagus officinalis*) dagegen zu dieser Zeit Amide in ebenso grosser Menge wie Aminosäure auftreten. Ich gehe jetzt zu meinen eigenen Versuchen über.

Meine Analysen wurden an Bulbillen und Knollen von *Begonia* durchgeführt; die ersten sammelte ich am 20. Okt. 1932 und hielt sie im Eisschrank, während die letzten vom freien Felde gesammelt wurden. Für die Analysen wurden, mit Ausnahme ganz weniger Fälle, wenigstens fünf oder sechs Individuum genommen.

Die Analysenergebnisse sind in Tabelle 4 zusammengestellt.

Aus diesen Ergebnissen kann man schliessen, dass bei den Knollen und Bulbillen der Gehalt an löslichem Stickstoff, im Gegensatz zu anderen Organen, viel grösser als der an Eiweiss-Stickstoff ist und etwa  $\frac{2}{3}$  des Gesamt-Stickstoffs ausmacht. Meine Ergebnisse stimmen mit denen SCHULZES gut überein und können als allgemeines Kennzeichen für unterirdische Reservestoffbehälter gelten. Nach meinen Ergebnissen kann dies auch auf die oberirdischen Bulbillen von *Begonia* ausgedehnt werden. Asparagin bzw. Glutamin, welches wir hier als „Amid-N“ anführen, spielt bei Bulbillen von *Begonia* scheinbar eine bedeutende Rolle. Der Beginn des Sprossens der Knospe aus den Knollen ist, im Gegensatz zu den Bulbillen, dadurch gekennzeichnet, dass hier die Aminosäuren eine durchaus herrschende Form des löslichen Stickstoffs darstellen (etwa  $\frac{2}{3}$  des löslichen Stickstoffs) und die Amide stark zurücktreten, sodass bei ihnen der prozentige Gehalt an Amino- und Amid-Stickstoff grade im umgekehrten Verhältnis steht. Der Ammon-Stickstoff ist bei Knollen wie Bulbillen, im Gegensatz zu anderen Organen von *Begonia*, ganz gering.

TABELLE 4.

Der Stickstoffgehalt der Bulbillen und Knollen von  
*Begonia Evansiana* ANDR.

Pflanzenteil		Bulbillum J	Bulbillum K	Bulbillum L	Knollen 1	Knollen 2	Knollen 3																								
Frischgewicht des Materials      mg		135.279	55.881	79.140	166.732	204.099	119.332																								
Gesamt-N      mg		0.6160	0.3850	0.6258	0.5852	0.6160	0.4170																								
Gesamt-N in % d. Frischgewichtes		0.45	0.68	0.79	0.35	0.30	0.37																								
N in % d. Gesamt-N	Eiweiss-N	23.09	36.73	32.22	39.24	38.63	37.98																								
	Lösl.-N	70.90	63.27	67.78	60.76	61.37	62.02																								
	Ammon-N	1.71	7.09	4.36	3.23	3.40	2.35																								
	Amid-N	53.18	39.27	44.59	12.20	17.04	14.09																								
	Amino-N	16.02	16.90	18.82	45.33	40.90	45.57																								
N in % d. Lösl.-N	Ammon-N	2.40	11.21	6.44	5.32	5.56	3.79																								
	Amid-N	75.00	62.07	65.79	20.08	27.78	22.73																								
	Amino-N	22.59	26.72	27.77	74.61	66.67	73.48																								
mg N in 1000 g Frischgewicht	Gesamt-N	4553.5	6889.6	7907.5	3509.8	3019.5	3745.8																								
	Eiweiss-N	1324.7	2530.4	2547.4	1377.0	1166.6	1422.9																								
	Lösl.-N	3228.8	4359.2	5360.1	2132.8	1852.9	2322.9																								
	Ammon-N	77.6	488.5	344.9	113.4	102.9	88.0																								
	Amid-N	2421.6	2705.7	3526.7	428.2	514.7	527.9																								
	Amino-N	729.6	1165.0	1488.5	1591.2	1235.3	1707.0																								
Bemerkung	Der Zustand der Bulbillen:				Der Zustand der Knollen:																										
	Länge      5-7 mm																														
	Breite      3-4 mm																														
	Knospe    1-1.5 mm																														
	Wurzel    0.5-1 mm																														
	Sie wurden am 19. April und am 25. April analysiert.				Sie wurden am 5. Mai analy- siert.																										
				<table><tr><td>Nr.</td><td>1</td><td>2</td><td>3</td></tr><tr><td>Knospe</td><td>10 mm</td><td>8 mm</td><td>5 mm</td></tr><tr><td>Wurzel</td><td>2-3 mm</td><td>2-3 mm</td><td>2-3 mm</td></tr><tr><td>Gewicht</td><td>ca. 2 g</td><td>ca. 1 g</td><td>ca. 1 g</td></tr><tr><td>Breite</td><td>20 mm</td><td>10 mm</td><td>10 mm</td></tr><tr><td>Höhe</td><td>15 mm</td><td>8 mm</td><td>8 mm</td></tr></table>				Nr.	1	2	3	Knospe	10 mm	8 mm	5 mm	Wurzel	2-3 mm	2-3 mm	2-3 mm	Gewicht	ca. 2 g	ca. 1 g	ca. 1 g	Breite	20 mm	10 mm	10 mm	Höhe	15 mm	8 mm	8 mm
Nr.	1	2	3																												
Knospe	10 mm	8 mm	5 mm																												
Wurzel	2-3 mm	2-3 mm	2-3 mm																												
Gewicht	ca. 2 g	ca. 1 g	ca. 1 g																												
Breite	20 mm	10 mm	10 mm																												
Höhe	15 mm	8 mm	8 mm																												

### C. Tagesschwankungen des N-Stoffwechsels in den Blättern.

Über den Nacht-N-Stoffwechsel der Blätter liegen bisher nur wenig und einander oft widersprechende Ergebnisse vor. SUZUKI untersuchte die nachts erfolgende Auswanderung einzelner Fraktionen N-haltiger Substanzen, die das Frischgewicht der Blätter betrafen. Er kam dabei zum

Schluss, dass nachts sowohl das Gesamt-Stickstoff als auch das Eiweiss-Stickstoff und das Asparagin-N eine Verminderung erfahren. Schnitt er die Blätter am Abend ab und liess sie in feuchter Atmosphäre 20–48 Stunden im Dunkeln liegen, so beobachtete er starken Eiweissabbau und bedeutende Vermehrung der Amide und auch der Aminosäuren. KOSUTANY beobachtete an *Vitis riparia*-Blättern betr. ihres Trockengewichts relative Zunahme des Gesamt- und Eiweiss-Stickstoffs und auch des Ammoniaks, völliges Verschwinden des Asparagins und Abnahme des löslichen Stickstoffs während der Nacht. Absolute Mengen lassen sich, weil Angaben über die benutzten Blattflächen fehlen, nicht errechnen.

Durch die Untersuchung der nachts erfolgenden Auswanderung von Stickstoff aus Blättern hat CHIBNALL anderseits festgestellt, dass die Beziehung der gefundenen Stickstoffwerte auf das Trockengewicht immer zu falschen Ergebnissen führen muss, da die Auswanderung N-haltiger und N-freier Stoffe in sehr verschiedenem Masse vor sich geht. Er ist der Ansicht, dass eine Beziehung auf das Frischgewicht vielleicht bei weitem richtigeren Ergebnissen führen würde. Von diesem Gesichtspunkt aus stellte er die Nacht-Abnahme des Gesamt- und des Eiweiss-Stickstoffs fest.

RUHLAND und WETZEL zeigten auf Grund ihrer Untersuchungen der Tagesschwankungen des N-Stoffwechsels von *Begonia semperflorens*, dass während der Nacht grössere Mengen von Aminosäuren aus den alten Blättern in die jungen Blätter abgeleitet werden. Der  $\text{NH}_3$ -Gehalt sinkt nachts in den alten ebenso wie in den jungen Blättern. Ammoniak wird allerdings in jungen Blättern zur Eiweissynthese verwendet oder in gewissen Fällen in Amide übergeführt.

MOTHES untersuchte vor kurzem den N-Stoffwechsel höherer Pflanzen und kam dabei zum Schluss, dass, mit Ausnahme des Ammoniaks, alle Stickstoff-Fractionen in den ausgewachsenen Blättern abnehmen. Unbedeutend ist die Abnahme des Eiweiss-Stickstoffs, bedeutend die des Amid-Stickstoffs. In den Einzelversuchen fand er grosse Schwankungen beim Rest-Stickstoff, während diese Veränderungen bei den nicht ausgewachsenen Blättern gar nicht oder nur in geringem Masse wahrzunehmen waren. Bei den abgeschnittenen Blättern nimmt der Eiweiss- und Ammon-Stickstoff morgens an Menge ab. Der lösliche Stickstoff nimmt zu, doch dabei der Amid-Stickstoff mehr als der Rest-Stickstoff. Ob diese Steigerung des Amid-Stickstoffs auf das Verschwinden des Ammon-Stickstoffs und zum Teil auf den hydrolytischen Abbau des Eiweisses zurückzuführen ist, oder ob er einem sekundären Vorgang seine Entstehung verdankt, ist schwer zu entscheiden.



GOUWENTAK studierte unlängst den N-Stoffwechsel von *Helianthus annuus* L. Dabei wurde die Blatthälftenmethode von SACHS benutzt. Es ergab sich folgendes: Im Gegensatz zu den Untersuchungen anderer Autoren überwiegt während der Nacht bald die Eiweisspaltung, bald der Eiweissaufbau. Es konnte noch nicht ermittelt werden, wovon das Überwiegen des Eiweissaufbaus oder des Eiweissabbaus abhängt.

Ganz kürzlich hat SATTLER durch Untersuchungen des Stoffwechsels immergrüner Pflanzen nachgewiesen, dass sich bei *Hedera Helix* weder bei Tag noch bei Nacht Zu- oder Abnahme im Gesamt- und Eiweiss-Stickstoff erkennen lässt, sowohl bezüglich der Trockensubstanz als auch der Blattfläche. Auffallend dagegen war bei *Ilex Aquifolium* die Zunahme des Gesamt-Stickstoffs am Tage. Diese dauert von April bis Juni und im September vom Morgen bis zum Mittag, während mittags Ableitung stattfindet. Im Juli und August findet sich Zunahme bis zum Abend und nachts Abwanderung. In den Wintermonaten bleibt der Wert tagsüber beständig oder sinkt etwas.

Eine ausführliche Zusammenstellung dieser Ergebnisse findet sich bei MOTHES und GOUWENTAK.

Gehen wir jetzt zu unseren Versuchen über. Zur Bestimmung der Tagesschwankungen des Stickstoffgehalts der Blattspreite analysierte ich ein Individuum (Nr. 21), das 88 Tage lang in Kultur gewesen war. Zur Bestimmung des Tagesstickstoffs entnahm ich das Material um 13–14 Uhr und zu der des Nachtstickstoffs um 18–19 Uhr. Die Blätter wurden, wie bei meinen Versuchen üblich, quer in zwei gleiche Teile durchschnitten. Der Sonnenaufgang bzw. -untergang an diesem Tage war um 5 Uhr 48 Minuten bzw. um 16 Uhr 51 Minuten. Die Ergebnisse sind in folgender Tabelle und Figur zusammengestellt. Aus diesen Zahlen ersieht man, dass der absolute Wert des Gesamt-Stickstoffs, auf 1000 g Frischgewicht bezogen, im Gegensatz zum Säuregehalt, im allgemeinen von der Basis nach der Spitze hin steigt. Der Gesamt-, der Eiweiss-, der lösliche und auch der Ammon-Stickstoff weisen im Verhältnis zum Frischgewicht des Materials nachts geringe Zunahme in den oberen, jungen Blattspreiten, in den mittleren geringe und in den unteren und alten starke Abnahme auf. Was das Verhältnis des Eiweiss- und des löslichen Stickstoffs zum Gesamt-Stickstoff betrifft, so kann man keinen merklichen Unterschied zwischen Tag und Nacht erkennen. In bezug auf das Verhältnis des Ammon-, Amid- und Amino-Stickstoffs zum löslichen Stickstoff kann man aber starke Zunahme des Ammon- und geringe Zunahme des Amid-Stickstoffs in der Nacht erkennen, während der Amino-Stickstoff stark

TABELLE 5.  
Tagesschwankungen des N-Gehalts in der Blattspreite von *Begonia Evansiana* ANDR.  
Nr. 21. 88tägige Züchtung im Gewächshaus.

Bemerkung	Tagessstickstoffbestimmung					Nachtsstickstoffbestimmung				
	I	II	III	IV	V	I	II	III	IV	V
Nr. d. Blattspreite von unten										
Frischgewicht d. Materials mg	139.303	306.903	381.303	539.735	97.719	219.777	314.340	596.769	661.591	132.438
Gesamt-N mg	0.4116	0.7819	1.5777	1.6562	0.2968	0.3360	0.6734	1.4938	2.0916	0.4046
Gesamt-N in % d. Frischgewichtes	0.29	0.25	0.41	0.30	0.30	0.15	0.21	0.25	0.31	0.30
N in % d. Gesamt-N	Eiweiss-N	83.67	88.71	91.63	83.02	85.00	85.65	90.44	92.17	84.43
	Lösl.-N	16.23	11.29	10.65	8.37	15.00	14.35	9.56	7.83	15.57
	Ammon-N	4.08	2.95	3.19	2.53	4.38	3.43	3.66	2.91	4.67
	Amid-N	6.17	4.83	4.79	3.80	6.25	5.47	4.78	3.82	6.23
	Amino-N	6.07	3.50	2.66	2.03	4.38	5.45	1.13	1.10	4.67
N in % d. Lösl.-N	Ammon-N	25.00	26.16	30.00	30.30	29.17	23.91	38.24	37.18	30.00
	Amid-N	37.79	42.81	45.00	45.45	41.67	38.09	50.00	48.72	40.00
	Amino-N	37.20	32.03	25.00	24.24	29.17	37.99	11.76	14.10	30.00
	Gesamt-N	2954.7	2547.7	4137.7	3068.6	1528.8	2142.3	2503.1	3161.5	3055.0
mg N in 1000 g Frischgewicht	Eiweiss-N	2472.3	2260.0	3697.1	2811.8	1299.5	1835.0	2263.8	2913.9	2579.3
	Lösl.-N	482.4	287.7	440.6	256.8	229.3	307.3	229.3	247.6	475.7
	Ammon-N	120.6	75.3	132.2	77.8	66.9	73.5	91.5	92.1	142.7
	Amid-N	182.3	123.1	198.3	116.7	95.5	117.0	119.6	120.6	190.3
	Amino-N	179.5	89.3	110.1	62.3	66.9	116.8	28.2	34.9	142.7
Amid/NH <sub>3</sub>	1.51	1.63	1.50	1.50	1.99	1.42	1.59	1.30	1.30	1.33

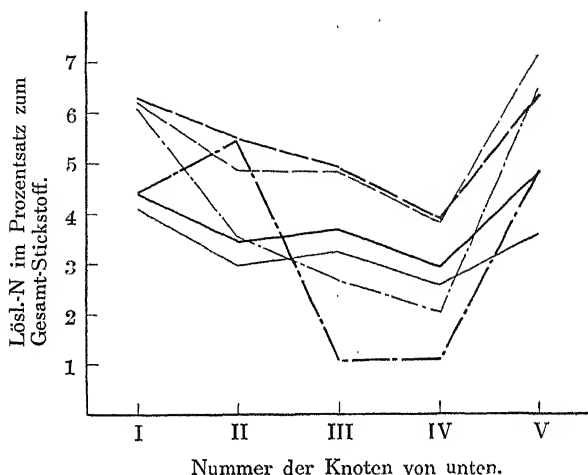


Fig. 3. Tagesschwankung des Stickstoffgehalts in der Blattspreite von *Begonia Evansiana* ANDR. Nr. 21. 88tägige Züchtung im Gewächshaus.

	Tageswerte	Nachtwerte
Ammon-N	————	————
Amid-N	—— ———	—— ———
Amino-N	— · — · —	— · — · —

abnimmt. Dieses Verhältnis gilt auch für die auf Gesamt-N bezogenen Stickstoffwerte. Der Quotient Amid/Ammon ist bei allen Blattspreiten in der Nacht kleiner als der am Tage; diese Tatsache zeigt die relative Zunahme des Ammon-Stickstoff in der Nacht. Bei älteren Blattspreiten der absolute Ammon-Stickstoffgehalt in der Nacht ist kleiner als der am Tage. Bei jüngeren Blattspreiten ist das Verhältnis grade umgekehrt.

Die Ergebnisse aus der Analyse eines anderen Individuums (Nr. 24), das 104 Tage lang in Kultur gewesen war, sind folgende. Aus Tabelle 6 und Fig. 4 und 5 geht hervor, dass der Gehalt an Eiweiss-Stickstoff im Verhältnis zum Gesamt-Stickstoff im allgemeinen in der Nacht etwas grösser als am Tage ist und dass beim löslichen Stickstoff das Verhältnis umgekehrt ist. Der Ammon- und der Amid-Stickstoff nimmt bei den oberen, jüngeren Blattspreiten in der Nacht zu und der Amino-Stickstoff deutlich ab. Dieselbe Beziehung kann man auch beim Ammon-, Amid- und Amino-Stickstoff im Verhältnis zum löslichen Stickstoff sehen. Die Gesamt-, Eiweiss- und Ammon-Stickstoffmenge, auf das Frischgewicht bezogen, nimmt bei diesem Material in den oberen Blattspreiten etwas zu, dagegen in den unteren in der Nacht stark zu. Der Amino-Stickstoff

TABELLE 6.  
Tagesschwankungen des N-Gehalts in der Blattspreite von *Begonia Evansiana* ANDR.  
Nr. 24. 104tägige Züchtung im Gewächshaus.

Bemerkung		Tagesstickstoffbestimmung					Nachtstickstoffbestimmung				
		I	II	III	IV	V	I	II	III	IV	V
Nr. d. Blattspreite von unten											
Frischgewicht d. Materials mg		181.640	444.542	407.736	771.551	437.011	183.992	482.091	551.895	612.007	525.789
Gesamt-N mg		0.3346	0.9940	0.9632	2.3842	1.3706	0.5256	1.2208	1.2810	1.6744	1.6986
Gesamt-N in % d. Frischgewichtes		0.18	0.22	0.24	0.31	0.31	0.29	0.25	0.23	0.27	0.32
N in % d. Gesamt-N	Eiweiss-N	78.66	85.21	83.43	93.83	87.74	80.03	88.99	87.21	89.97	89.85
	Lös.-N	21.34	14.79	16.57	6.17	12.26	19.97	11.01	12.79	10.03	10.15
	Ammon-N	6.84	2.75	4.14	0.96	1.84	5.19	3.61	4.92	3.26	1.86
	Amid-N	11.29	5.07	6.26	1.16	1.94	5.59	5.16	5.25	4.52	3.22
	Amino-N	3.19	6.97	5.89	4.01	8.48	9.19	2.24	2.62	2.26	5.07
N in % d. Lös.-N	Ammon-N	33.07	18.57	25.00	15.58	15.00	26.00	32.81	34.46	32.50	18.29
	Amid-N	53.94	34.29	39.47	18.09	15.83	28.00	46.88	41.03	45.00	31.71
	Amino-N	14.99	47.14	35.53	66.33	69.17	46.00	20.31	20.51	22.50	50.00
	Gesamt-N	1842.1	2236.0	2862.3	3090.2	3136.3	2857.7	2532.3	2321.1	2735.9	3227.0
N in 1000 g Frischgewicht	Eiweiss-N	1449.0	1905.3	1970.9	2899.7	2751.9	2287.1	2253.5	2034.3	2461.4	2899.6
	Lös.-N	393.1	330.7	391.4	190.5	384.4	570.6	278.8	296.8	274.5	327.5
	Ammon-N	126.1	61.4	97.8	29.7	57.7	148.3	91.5	114.1	89.2	59.9
	Amid-N	208.1	113.4	154.5	34.5	60.8	159.8	130.7	121.8	133.5	103.8
	Amino-N	58.9	155.9	139.1	126.3	265.9	262.5	56.6	60.9	61.8	163.8
Amid/NH <sub>3</sub>		1.65	1.84	1.57	1.16	1.05	1.07	1.42	1.06	1.39	1.73

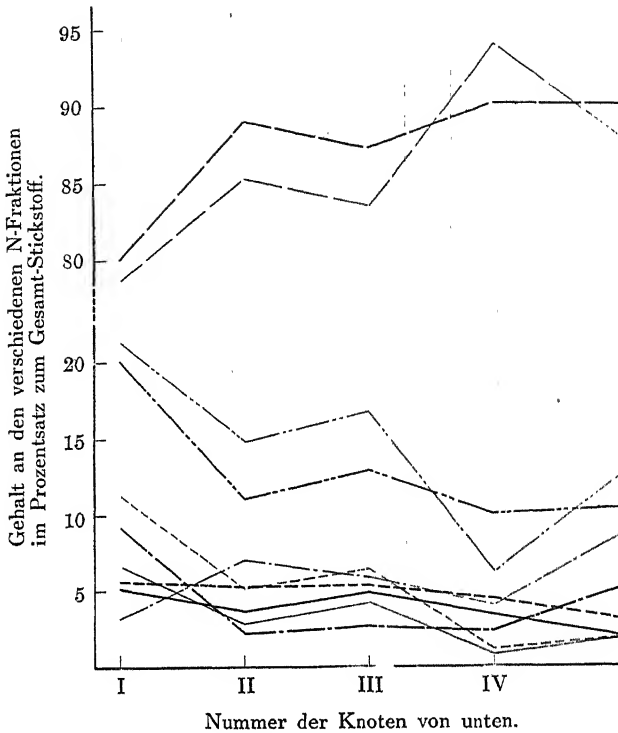


Fig. 4. Tagesschwankung des Stickstoff-Gehalts in der Blattspreite von *Begonia Evansiana* ANDR. Nr. 24. 104tägige Züchtung im Gewächshaus.

— — — — —	Nacht	} Eiweiss-N	— — — — —	Nacht	} Ammon-N
— — — — —	Tag		— — — — —	Tag	
— · — · — · —	Nacht	} Lössl.-N	— · — · — · —	Nacht	} Amid-N
— · — · — · —	Tag		— · — · — · —	Tag	
— — — — —	Nacht	} Amino-N	— — — — —	Nacht	} Amino-N
— · — · — · —	Tag		— · — · — · —	Tag	

nimmt durchgehends in der Nacht deutlich ab. Der Quotient Amid/Ammon bei den meisten älteren Blattspreiten ist in der Nacht stets kleiner als am Tage, sodass auf nächtliche, relativ geringere Abnahme oder sogar Zunahme des Ammon-Stickstoffs geschlossen werden muss.

#### D. Zusammenhang zwischen dem Säure- und dem N-Stoffwechsel.

##### 1. Säure- und Stickstoffgehalt in den Blütenblättern.

SCHUMACHER hat nachgewiesen, dass bei Kakteen der Höhepunkt ihrer synthetischen Entwicklung bereits überschritten ist, wenn sich die Blüte

aus der Knospe entfaltet. Der Eiweissaufbau ist beim Aufblühen schon beendet, und im Innern spaltet sich fortwährend Eiweiss ab, und die Blüte beginnt bei einem gewissen Punkt plötzlich und unaufhaltsam zu welken und geht zugrunde. Seiner Meinung nach ist dieser Vorgang für die Familie der Kakteen charakteristisch. Er hat auch nachgewiesen, dass in den Orchideenblütenblättern eine bestimmte Zeit nach der Bestäubung, etwa vom Beginn der Narbenschwellung ab, ein intensiver Eiweissabbau erfolgt, der bis zu den ersten, äusserlich sichtbaren Verwelkungserscheinungen durchschnittlich etwa 25% des Gesamteiweisses erfasst. Aus seinen Versuchen geht auch folgendes: Bei ephemeren Blüten erfolgt die Zertrümmerung der Eiweisse nach einem ohne Eiweissvermehrung erfolgenden starken Flächenwachstum in den Nachmittagsstunden explosionsartig, wobei eine ungeheuere Intensität des Stoffumsatzes erreicht wird.

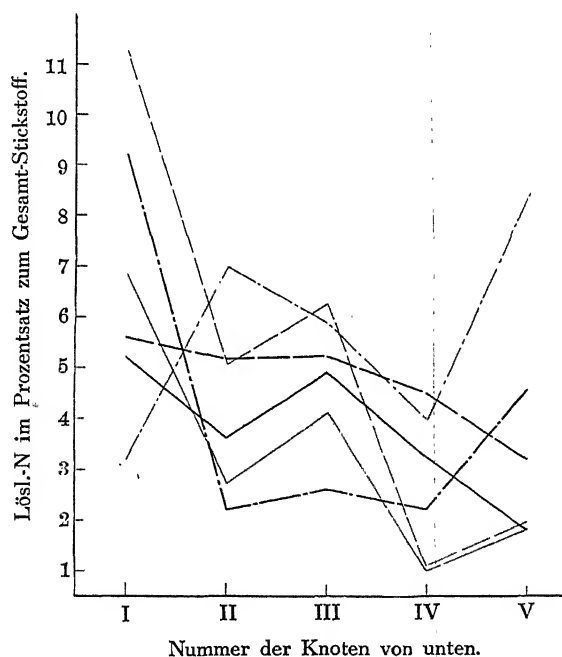


Fig. 5. Tagesschwankungen des Stickstoffgehalts in der Blattspreite von *Begonia Evansiana* ANDR. Nr. 24. 104tägige Züchtung im Gewächshaus.

	Tageswerte	Nachtwerte
Ammon-N	— — — — —	— — — — —
Amid-N	- - - - -	- - - - -
Amino-N	- · - · - ·	- · - · - ·

Um das Verhältnis zwischen dem Säure- und dem Stickstoffgehalt an ein und derselben Blüte (hauptsächlich an der männlichen Blüte) von *Begonia Evansiana* ANDR. zu erforschen, wurden die Analysen durchgeführt, deren Ergebnisse in Tabelle 7 wiedergegeben sind. Um diese Verhältnisse anschaulich zu machen, sind die Ergebnisse auch graphisch dargestellt worden (Fig. 6). Ein Blick auf die Figur zeigt, dass der Säuregehalt der halbentwickelten Blüte am grössten und der der Blüten-

TABELLE 7.  
Der Säure- und Stickstoffgehalt in der Blüte von  
*Begonia Evansiana* ANDR.

Pflanzenteil		Vollständig ent- wickelte Blüte. (7 Blüten)	Halbentwickelte Blüte (7 Blüten)	Blütenknospen (5 Bk.)
Frischgewicht des Materials (mg)		770.115	651.734	803.408
Oxalsäure mg		5.833	5.170	5.733
Prozent		0.76	0.79	0.71
mg Oxalsäure in 1000 g Frischgew.		7574.1	7932.6	7135.8
Frischgewicht des Materials (mg)		823.250	686.643	820.314
Gesamt-N. mg		1.4860	1.2124	1.5190
Gesamt-N in % d. Frischgewichtes		0.18	0.18	0.19
N in % d. Gesamt-N	Eiweiss-N	73.49	80.60	83.69
	Lösl.-N	26.51	19.40	16.31
	Ammon-N	11.59	8.73	7.74
	Amid-N	7.91	5.89	5.53
	Amino-N	7.01	4.78	3.17
N in % d. Lösl.-N	Ammon-N	43.71	44.99	47.46
	Amid-N	29.85	30.36	33.89
	Amino-N	26.44	24.65	19.45
mg N in 1000 g Frischgewicht	Gesamt-N	1805.0	1765.6	1851.7
	Eiweiss-N	1326.4	1423.1	1549.6
	Lösl.-N	478.6	342.5	302.1
	Ammon-N	209.2	154.1	143.4
	Amid-N	142.8	103.9	99.9
	Amino-N	126.6	84.5	58.8
Amid/NH <sub>3</sub>		0.682	0.674	0.696

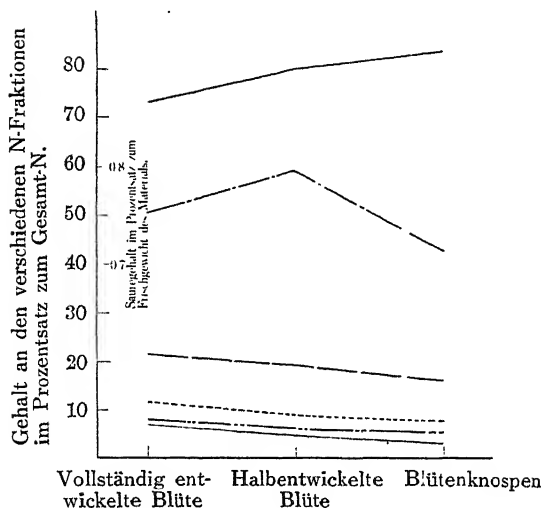


Fig. 6. Säure- und Stickstoffgehalt der Blüten von *Begonia Evansiana* ANDR.

———— Eiweiss-N      ———— Lösl.-N      ..... Säure  
 - - - - - Ammon-N      - · - · - Amid-N      ——— Amino-N

knospen am kleinsten ist, was mit den Ergebnissen in meiner vorigen Mitteilung übereinstimmt. Dies ist sicher auf die verschiedene Intensität des Stoffumsatzes zurückzuführen.

Was zunächst das Verhältnis des Eiweiss-Stickstoffs zum Gesamt-Stickstoff betrifft, so ist es bei den Blütenknospen am grössten und bei den vollständig entwickelten Blüten am kleinsten. In bezug auf das des löslichen Stickstoffs bzw. dessen Fraktionen ist es gerade umgekehrt. Trotzdem ist das Verhältnis des Ammon- und des Amid-Stickstoffwerts zum gesamten löslichen Stickstoff bei Blütenknospen am grössten und bei vollständig entwickelten Blüten am kleinsten; aber beim Amino-Stickstoff ist es umgekehrt (Tabelle 7). Die Reihenfolge der absoluten N-Menge in bezug auf 1000 g Frischgewicht ist, wie folgt:

Beim Gesamt-Stickstoff: Blütenknospen > Vollständig entwickelte B. > Halbentwickelte B.

Beim Eiweiss-Stickstoff: Blütenknospen > Halbentwickelte B. > Vollständig entwickelte B.

Beim löslichen Stickstoff: Vollständig entwickelte B. > Halbentwickelte B. > Blütenknospen.

Beim Ammon-Stickstoff: Vollständig entwickelte B. > Halbentwickelte B. > Blütenknospen.



Beim Amid-Stickstoff: Vollständig entwickelte B. > Halbentwickelte B. >  
Blütenknospen.

Beim Amino-Stickstoff: Vollständig entwickelte B. > Halbentwickelte B. >  
Blütenknospen.

Der Quotient Amid/Ammon blieb bei allen Blüten beinahe beständig, und Ammoniak überwiegt deutlich das Amid. Dies kann, oberflächlich betrachtet, zugunsten der Entstehung der Oxalsäure bei der Desaminierung der Aminosäuren sprechen. Doch die Mengenverhältnisse der Oxalsäure gehen nicht immer mit denen des Ammon-Stickstoffs parallel. Ob dabei das gefundene Ammoniak zum Teil von anderen Organen befördert worden ist, das bleibt noch weiterer Forschung überlassen.

## 2. Säure- und Stickstoff-Stoffwechsel in den einzelnen Pflanzenteilen.

1926 suchten RUHLAND und WETZEL festzustellen, ob die in *Begonia* nachweisbare Oxalsäure aus dem Eiweiss- oder dem Kohlenhydratstoffwechsel entsteht. Nach ihnen ging reichliche Oxalsäurebildung mit starker Anhäufung von Ammoniak einher. Bei ihrem Aufhören pflegte auch die Ammoniakbildung zugunsten der Aminosäure aufzuhören. Die Säure spielt eine Rolle als Entgifter des auftretenden Ammoniaks.

Deshalb kamen sie zum Schluss, dass das Auftreten der Oxalsäure aufs engste mit dem Eiweisstoffwechsel verknüpft.

Bei verschiedenen Pflanzen untersuchte KULTSCHER die Beziehungen zwischen dem N-Stoffwechsel und der Wasserstoffionenkonzentration des Zellsafts und stellte dabei fest, dass zwischen  $\text{NH}_3$  und dem Amid in der Pflanze ein Gleichgewicht besteht, welches in hohem Masse, wenn auch durchaus nicht ausschliesslich, vom pH des Zellsafts beeinflusst wird.

### Versuch 1.

Bei diesem Versuch wollte ich in ein und demselben einzelnen Pflanzenteile das Verhältnis zwischen dem Säure- und dem Stickstoffgehalt klarstellen. Das dazu bestimmte Individuum (Nr. 12) war 55 Tage lang in Kultur gewesen. Das erste Blatt, welches sekundär neu entwickelt war, und das 7te Blatt waren aber nicht gross genug, um die Bestimmung des Säure- sowie auch des Stickstoffgehalts nebeneinander durchzuführen, sodass ich bei ihnen immer nur einen von ihnen bestimmte.

Die Versuchsergebnisse sind in Tabelle 8 in üblicher Weise zusammengestellt. Aus Fig. 7 ist ersichtlich, dass der Säuregehalt des Blattstiels grösser als der der Blattspreite ist, während er im Stengel bedeutend

TABELLE 8.

Der Säure- und Stickstoffgehalt in den verschiedenen Pflanzenteilen  
von *Begonia Evansiana* ANDR. Nr. 12. 55tägige  
Züchtung im Gewächshaus.

(a) Blattspreite.

Nr. d. Blattspreite von unten		I *	II	III	IV	V	VI	VII
Frischgewicht d. Materials mg			207.764	183.343	120.762	1000.046	127.367	153.261
Oxalsäure mg			0.937	0.656	0.468	0.372	0.385	0.383
Prozent			0.45	0.35	0.38	0.37	0.30	0.24
mg Oxalsäure in 1000 g Frischgew.			4519.5	3577.9	3875.3	3718.2	3022.7	2499.0
Frischgewicht d. Materials mg		165.311	309.695	379.626	400.957	409.280	499.085	169.177
Gesamt-N mg		0.2856	0.5670	0.6776	0.7028	0.6972	0.9590	0.4158
Gesamt-N in % d. Frischgewichtes		0.17	0.18	0.18	0.18	0.17	0.19	0.25
N in % d. Gesamt-N	Eiweiss-N	51.47	77.78	72.11	73.71	75.90	76.79	62.63
	Lös.-N	48.53	22.22	27.89	26.29	24.10	23.21	37.37
	Ammon-N	5.88	5.19	4.32	2.79	3.01	5.26	5.56
	Amid-N	30.88	11.85	12.39	10.16	13.25	14.02	15.15
	Amino-N	11.77	5.19	10.54	13.75	6.39	3.94	15.37
N in % d. Lös.-N	Ammon-N	12.12	28.02	17.78	9.09	12.50	22.64	14.87
	Amid-N	63.64	53.33	44.44	38.64	55.00	60.38	40.54
	Amino-N	24.24	18.65	37.78	52.27	32.50	16.98	44.59
mg N in 1000 g Frischgewicht	Gesamt-N	1727.7	1830.8	1784.9	1552.8	1703.5	1921.5	2457.8
	Eiweiss-N	889.3	1424.0	1287.1	1291.9	1293.0	1475.5	1539.2
	Lösl.-N	838.4	406.8	497.8	460.9	410.5	446.0	918.6
	Ammon-N	101.6	94.9	88.5	41.9	51.3	101.0	136.5
	Amid-N	533.5	217.0	221.3	178.1	225.7	269.3	372.4
	Amino-N	203.3	94.9	188.0	240.9	109.0	75.7	409.7
Amid/Ammon		5.25	2.28	2.50	4.17	4.39	2.66	2.72

\* Blattspreite einschliesslich des betreffenden Blattstiels.

## (b) Blattstiel.

Nr. d. Blattstiels von unten		II	III	IV	V	VI
Frischgewicht d. Materials mg		57.902	84.490	77.241	84.545	34.517
Oxalsäure mg		0.299	0.399	0.357	0.361	0.121
Prozent		0.51	0.47	0.46	0.42	0.35
mg Oxalsäure in 1000 g Frischgew.		5163.8	4722.4	4621.8	4269.9	3505.5
Frischgewicht d. Materials mg		144.417	222.071	313.847	151.064	499.085
Gesamt-N mg		0.2296	0.2590	0.2646	0.2660	0.9590
Gesamt-N in % d. Frischgewichtes		0.15	0.11	0.08	0.17	0.19
N in % d. Gesamt-N	Eiweiss-N	26.83	43.24	38.09	32.11	29.29
	Lösl.-N	73.17	56.76	61.91	67.89	70.71
	Ammon-N	16.46	18.65	19.84	25.26	23.57
	Amid-N	34.76	27.57	31.75	31.58	30.00
	Amino-N	21.95	10.54	10.32	11.05	17.65
N in % d. Lösl.-N	Ammon-N	22.50	32.86	32.05	37.21	33.33
	Amid-N	47.50	48.57	51.28	46.51	42.42
	Amino-N	30.00	18.57	16.67	16.28	24.24
mg N in 1000 g Frischgewicht	Gesamt-N	1589.8	1166.3	843.1	1760.8	2722.3
	Eiweiss-N	426.5	504.3	321.2	565.3	797.2
	Lös.-N	1163.3	662.0	521.9	1195.5	1925.1
	Ammon-N	261.7	217.5	167.3	444.8	641.7
	Amid-N	552.6	521.5	267.6	556.1	816.7
	Amino-N	349.0	123.0	87.0	194.6	466.7
Amid/Ammon		2.11	2.39	1.59	1.27	1.27

(c) Knollen und Stengel einschliesslich der betreffenden Knoten.

Pflanzenteil		Knollen	I Stengel	II Stengel	III Stengel	IV Stengel	V Stengel	VI Stengel	VII Stengel
Frischgewicht d. Materials mg		231.210	111.506	75.449	125.361	91.471	46.514	115.231	103.621
Oxalsäure mg		0.068	0.216	0.161	0.295	0.226	0.112	0.339	0.441
Prozent		0.02	0.19	0.21	0.23	0.24	0.24	0.29	0.42
mg Oxalsäure in 1000 g Frischgew.		294.1	1937.1	2133.8	2353.2	2470.7	2407.8	2941.9	4255.8
Frischgewicht d. Materials mg		226.079	425.261	236.239	395.539	251.937	230.524	141.070	
Gesamt-N mg		0.6006	0.3794	0.2800	0.4144	0.3080	0.3276	0.3038	
Gesamt-N in % d. Frischgew.		0.26	0.08	0.11	0.10	0.12	0.14	0.21	
N in % d. Gesamt-N	Eiweiss-N	26.57	52.40	47.50	39.19	38.64	39.74	37.79	
	Lösl.-N	73.43	47.60	52.50	61.81	61.36	60.26	62.21	
	Ammon-N	4.19	16.50	18.00	21.79	15.00	16.03	18.66	
	Amid-N	30.77	22.14	28.50	30.41	30.00	29.49	26.59	
	Amino-N	38.46	8.86	6.00	8.62	16.36	14.74	16.95	
N in % d. Lösl.-N	Ammon-A	5.71	34.88	34.29	35.83	24.44	26.59	30.00	
	Amid-N	41.91	46.51	54.29	50.00	48.89	48.94	42.75	
	Amino-N	52.38	18.61	11.42	14.17	26.67	24.47	27.25	
mg N in 1000 g Frischgewicht	Gesamt-N	2656.6	892.2	1185.2	1047.7	1222.5	1421.1	2153.5	
	Eiweiss-N	705.9	467.5	563.0	410.6	472.3	564.8	813.8	
	Lösl.-N	1950.7	424.7	622.2	637.1	750.2	856.3	1339.7	
	Ammon-N	111.5	148.1	213.3	228.3	183.4	227.8	401.9	
	Amid-N	817.4	197.5	337.8	318.5	366.7	419.0	572.8	
	Amino-N	1021.8	79.0	71.1	90.3	200.1	209.5	365.0	
Amid/Ammon		7.33	1.33	1.58	1.39	1.99	1.83	1.42	

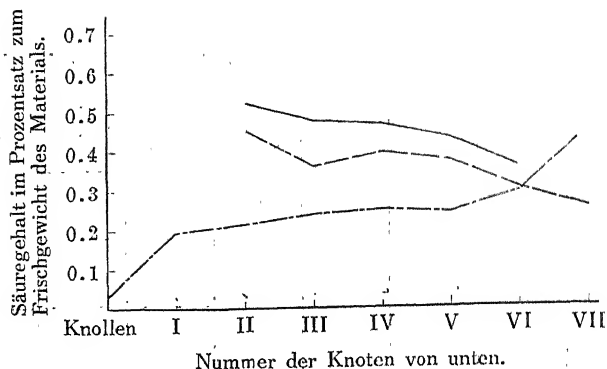


Fig. 7. Der Säuregehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. 12. 55tägige Züchtung im Gewächshaus.

— Blattstiel. — Blattspreite.  
 - - - Stengel einschliesslich der betreffenden Knoten.

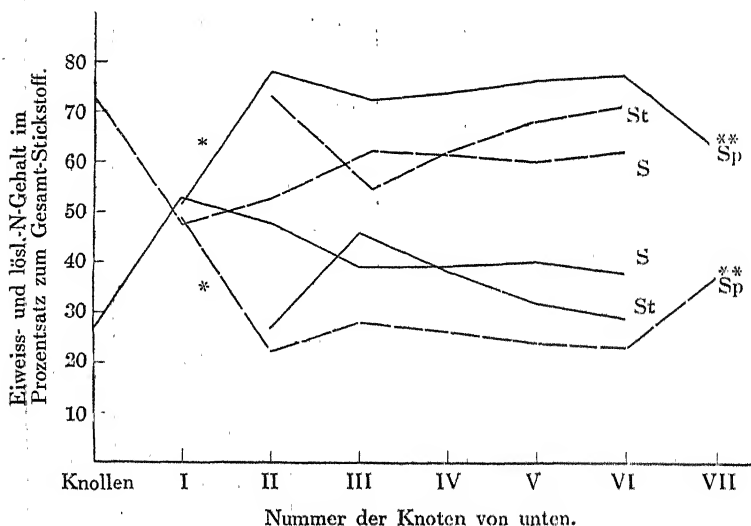


Fig. 8. Eiweiss- und lösli.-N-Gehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. 12. 55tägige Züchtung im Gewächshaus.

— Eiweiss-N \* sekundär entwickelte Blattspreite einschliesslich des betreffenden Blattstiels.  
 - - - Lösli.-N \*\* Blattspreite einschliesslich des betreffenden Blattstiels.

St: Blattstiel. Sp: Blattspreite.  
 S: Stengel einschliesslich der betreffenden Knoten.

geringer als in den Blättern ist. Sowohl bei den Blattspreiten als auch den Blattstielen ist allgemein die Neigung zur Abnahme des Säuregehalts nach der Reihenfolge der Knoten von der Basis nach der Spitze wahrzunehmen. Beim Stengel, einschliesslich der betreffenden Knoten, ist das Verhältnis umgekehrt. Bei den Knollen kann man nur sehr geringe Menge von Säure nachweisen. Darin stimmt das Ergebnis mit dem der vorigen Mitteilung gut überein.

In bezug auf das Verhältnis des Eiweiss- und des löslichen Stickstoffs zum Gesamt-Stickstoff ist beachtenswert, dass bei den Blattspreiten der Eiweiss-Stickstoff den löslichen Stickstoff überwiegt. Das Verhältnis fällt für die Blattstiele und die Stengel einschliesslich der betreffenden Knoten gerade umgekehrt aus.

In den Knollen findet sich sehr reichlich löslicher Stickstoff (Fig. 8). Dieses Ergebnis spricht dafür, dass der lösliche Stickstoff in den unterirdischen Reservestoffbehälter je nachdem einen ansehnlichen Teil des Gesamt-Stickstoffs ausmacht, ganz wie das bei anderen Pflanzen auch der Fall ist.

Der prozentuale Anteil der Fraktionen der löslichen N-Verbindungen ist, soweit meine Versuche reichen, je nach dem verschiedenen Teile der Pflanze sehr verschieden, jedoch macht er nicht weniger als ein Drittel des Gesamt-Stickstoffs aus und erreicht bei unseren Begonien sogar 73%. Die erste und die 7te Blattspreite enthielten eine verhältnismässig geringe Menge von Eiweiss-Stickstoff. Das kann darauf beruhen, dass sie mit dem betreffenden Blattstiele, der eine grosse Menge löslichen Stickstoff enthielt, zusammen analysiert wurden.

Die Beziehungen zwischen den löslichen Fraktionen der N-Verbindungen, namentlich dem Ammon-, Amid- und Amino-Stickstoff, zum Gesamt-Stickstoff in den einzelnen Teilen des Untersuchungsmaterials ist in Fig. 9 graphisch dargestellt. Aus dieser Figur ist zu entnehmen, dass sich bei den Blattspreiten sehr wenig Ammon-Stickstoff findet, der keinen nennenswerten Schwankungen unterliegt, während der Gehalt an Amid-Stickstoff beinahe doppelt so gross wie der an Ammon-Stickstoff ist. Ebenso findet sich bei den Blattstielen mehr Amid-Stickstoff als Ammon-Stickstoff, und die Schwankungen bei jenem gehen beinahe parallel mit denen beim Ammon-Stickstoff, da diese beiden Fraktionen löslichen Stickstoffs der Reihenfolge der Knoten nach von der Basis nach der Spitze hin allmählich zunehmen.

Bei dem Stengel einschliesslich der betreffenden Knoten ist der Gehalt an Ammon-Stickstoff in den untersten Knollen am kleinsten und nimmt allmählich nach oben hin zu, während der Amid-Stickstoffgehalt, ganz wie

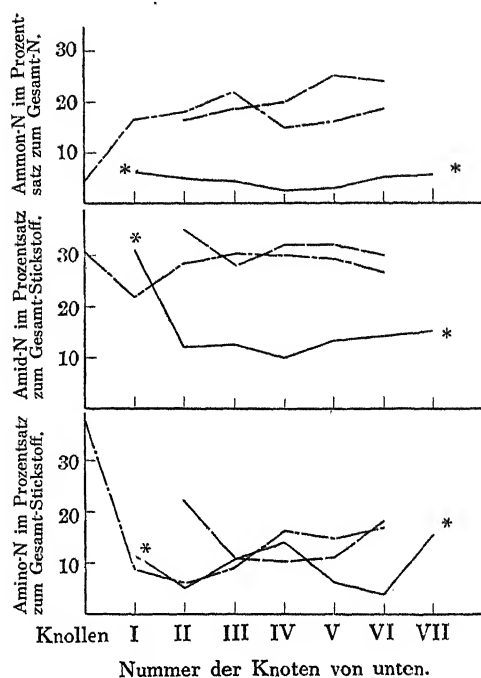


Fig. 9. Lösli-N-Gehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. 12. 55tägige Züchtung im Gewächshaus.

— Blattspreite                      — Blattstiel  
 — Stengel einschliesslich betreffender Knoten.  
 \* Blattspreite einschliesslich des betreffenden Blattstiels.

bei den Blattstielen, grösser als der des Ammon-Stickstoffs ist und mit den Schwankungen des Ammon-Stickstoffs parallel geht.

Der Gehalt an Amino-Stickstoff ist bei den Knollen am grössten und beträgt etwa 38.5% des Gesamt-Stickstoffs. Er sinkt aber beim Übergang zum Stengel bis auf 9% und steigt dann allmählich wieder bis zum gleichen Wert des Ammon-Stickstoffs.

Was die Beziehungen des Ammon-, Amid- und Amino-Stickstoffs zum löslichen Stickstoff anbetrifft, so kann man aus Tabelle 8 entnehmen, dass der Amid-Stickstoff etwa die Hälfte des löslichen Stickstoffs ausmacht und mit den Schwankungen des Ammon-Stickstoffs parallel geht. Die Schwankungen des Amino-Stickstoffgehalts bei den verschiedenen Organen sind sehr gross und unregelmässig.

In bezug auf den Gehalt des Gesamt-Stickstoffs bei 1000 g Frischgewicht habe ich gefunden, dass er bei Blattspreiten am grössten, bei

Blattstielen mässig und beim Stengel einschliesslich der betreffenden Knoten am kleinsten ist. Dagegen ist der Gehalt an löslichem Stickstoff im Blattstiel und Stengel einschliesslich der Knoten grösser und in der Blattspreite geringer. Unter den Fraktionen der löslichen N-Verbindungen ist der Ammon-Stickstoff im Blattstiel am reichsten und im Stengel mässig enthalten, während sich bei Blattspreiten, im Gegensatz zu ihrem Säuregehalt, nur wenig findet. Dementsprechend ist der Quotient Amid/Ammon beim Blattstiel am kleinsten und bei den Blattspreiten am grössten.

### Versuch 2.

Ein ganz ähnliches Verhältnis des Säuregehalts findet sich beim anderen Individuum (Nr. IV), welches 70 Tage lang gezüchtet und schon zur Blüte gekommen war (Tabelle 9). Fig. 10 gibt den prozentualen Säuregehalt

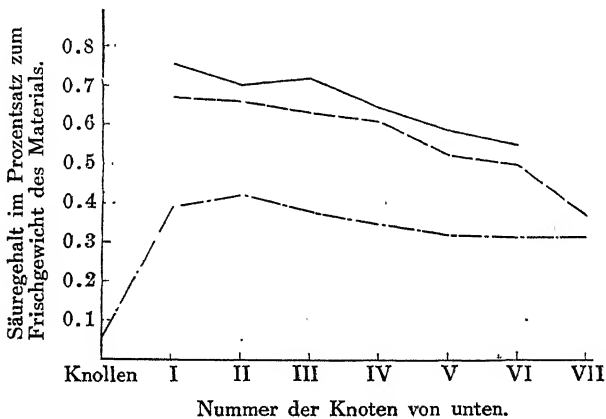


Fig. 10. Der Säuregehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. IV. 70tägige Züchtung im Gewächshaus.  
 ————— Blattstiel.      - - - - - Blattspreite.  
 - · - · - · - Stengel einschliesslich der betreffenden Knoten.

wieder. Dieser fällt hier viel grösser als beim vorigen Individuum aus. Trotzdem bleibt die Reihenfolge der Grösse des prozentualen Säuregehalts ganz dieselbe wie beim vorigen Falle.

Was die Beziehung des Eiweiss- und des löslichen Stickstoffs zum Gesamt-Stickstoff bei den Blattspreiten anbetrifft, so macht der Eiweiss-Stickstoff etwa 90% des Gesamt-Stickstoffs aus. Beim Blattstiel ist er geringer als der lösliche Stickstoff.

Beim Stengel einschliesslich der betreffenden Knoten ist er in den



TABELLE 9.

Der Säure- und Stickstoffgehalt in den verschiedenen Pflanzenteilen  
von *Begonia Evansiana* ANDR. Nr. IV. 70tägige  
Züchtung im Gewächshaus.

(a) Blattspreite.

Nr. d. Blattspreite von unten		I	II	III	IV	V	VI	VII *
Frischgewicht d. Materials mg		187.854	211.711	264.361	278.310	263.942	354.878	89.752
Oxalsäure mg		1.266	1.394	1.673	1.708	1.387	1.786	0.336
Prozent		0.67	0.65	0.63	0.61	0.52	0.50	0.37
mg Oxalsäure in 1000 g Frischgew.		6739.2	6584.4	6328.4	6136.5	5254.9	5032.7	3743.6
Frischgewicht d. Materials mg		526.722	546.487	657.366	675.916	586.933	596.407	220.137
Gesamt-N		1.0080	0.7294	0.9330	1.4020	1.1970	1.3594	0.5992
Gesamt-N in % d. Frischgewichtes		0.19	0.13	0.14	0.20	0.20	0.22	0.27
N in % d. Gesamt-N	Eiweiss-N	87.50	86.76	87.05	89.22	85.26	87.95	77.57
	Lösl.-N	12.50	13.24	12.95	10.78	14.74	12.05	22.43
	Ammon-N	1.46	2.59	1.51	0.89	2.98	2.78	8.76
	Amid-N	4.17	8.64	8.63	5.39	5.26	4.63	9.81
	Amino-N	6.86	2.02	2.81	4.49	6.49	4.63	3.86
N in % d. Lösl.-N	Ammon-N	11.67	19.56	11.67	8.33	20.24	23.08	39.06
	Amid-N	33.33	65.22	66.67	50.00	35.71	38.46	43.75
	Amino-N	55.00	15.22	21.67	41.67	44.05	38.46	17.19
mg N in 1000 g Frischgewicht	Gesamt-N	1913.7	1334.7	1480.1	2074.2	2039.4	2279.3	2690.1
	Eiweiss-N	1674.5	1157.9	1288.4	1850.5	1738.9	2004.7	2111.4
	Lösl.-N	239.2	176.8	191.7	223.7	300.5	274.7	610.5
	Ammon-N	27.9	34.6	22.4	18.7	60.8	63.5	238.5
	Amid-N	79.7	115.3	127.8	111.8	107.3	105.6	267.1
	Amino-N	131.6	26.9	41.5	93.2	132.4	105.6	104.9
Amid/Ammon		2.85	3.33	5.70	5.97	1.76	1.66	1.11

\* Blattspreite einschliesslich des betreffenden Blattstiels.

## (b) Blattstiel.

Nr. d. Blattstiels von unten		I	II	III	IV	V	VI
Frischgewicht d. Materials mg		178.690	98.716	232.671	416.675	269.027	113.823
Oxalsäure mg		1.349	0.695	1.683	2.704	1.578	0.630
Prozent		0.75	0.70	0.72	0.64	0.58	0.55
mg Oxalsäure in 1000 g Frischgew.		7549.3	7040.3	7233.3	6489.4	5865.3	5534.9
Frischgewicht d. Materials mg		610.627	178.165	383.099	963.306	558.556	299.470
Gesamt-N mg		0.5134	0.1610	0.3318	0.7112	0.5336	0.4452
Gesamt-N in % d. Frischgewichtes		0.10	0.09	0.08	0.07	0.95	0.14
N in % d. Gesamt-N	Eiweiss-N	39.27	45.22	50.63	45.08	44.34	39.62
	Lösl.-N	60.73	54.78	49.37	54.92	55.66	60.38
	Ammon-N	22.91	22.17	24.23	33.37	31.48	34.91
	Amid-N	12.27	18.26	18.92	13.58	18.10	14.16
	Amino-N	25.56	14.35	11.21	7.97	6.07	11.41
N in % d. Lösl.-N	Ammon-N	37.72	40.48	49.08	60.75	56.57	57.81
	Amid-N	20.20	28.00	28.21	24.73	32.52	23.44
	Amino-N	42.08	31.52	22.71	14.52	10.91	19.75
mg N in 1000 g Frischgewicht	Gesamt-N	840.8	903.7	866.1	738.3	955.3	1486.6
	Eiweiss-N	330.1	408.6	438.5	332.8	423.6	589.0
	Lösl.-N	510.6	495.1	427.6	405.5	531.7	897.6
	Ammon-N	192.6	200.4	209.9	246.3	300.8	518.9
	Amid-N	103.2	165.0	120.6	100.3	172.9	210.4
	Amino-N	214.8	129.7	97.1	58.9	58.0	168.3
Amid/Ammon		0.53	0.82	0.57	0.41	0.57	0.41

## (c) Knollen und Stengel einschliesslich der betreffenden Knoten.

Pflanzenteil	Knollen	I Stengel	II Stengel	III Stengel	IV Stengel	V Stengel	VI Stengel	VII Stengel
Frischgewicht d. Materials mg	270.619	435.578	274.690	247.403	253.893	251.894	229.812	149.839
Oxalsäure mg	0.171	1.695	1.157	0.938	0.884	0.811	0.726	0.474
Prozent	0.06	0.38	0.42	0.37	0.34	0.32	0.31	0.31
mg Oxalsäure in 1000 g Frischgew.	632.3	3891.3	4212.0	3791.3	3481.7	3219.6	3159.1	3163.3
Frischgewicht d. Materials mg	570.391	1092.542	1139.284	1181.514	1024.302	1038.520	850.340	556.893
Gesamt-N mg	1.1256	0.6860	1.0766	1.3104	1.2614	1.0816	1.0836	0.7864
Gesamt-N in % d. Frischgew.	0.12	0.06	0.09	0.11	0.12	0.10	0.12	0.14
N in % d. Gesamt-N	Eiweiss-N	39.18	68.78	62.16	53.53	48.07	45.64	53.10
	Lösl.-N	60.82	31.22	37.84	46.47	51.93	54.36	46.89
	Ammon-N	2.24	13.16	21.26	24.05	29.80	27.76	25.77
	Amid-N	5.59	12.86	10.14	11.22	10.99	14.37	13.18
	Amino-N	52.99	5.20	6.45	11.20	11.46	12.23	7.96
N in % d. Lösl.-N	Ammon-N	3.68	44.22	56.19	51.76	57.38	51.07	54.94
	Amid-N	9.20	41.18	26.80	24.13	21.16	26.43	28.10
	Amino-N	87.12	14.60	17.01	24.11	21.46	22.50	16.96
mg N in 1000 g Frischgewicht	Gesamt-N	1973.4	627.9	945.0	1109.1	1231.5	1041.5	1274.3
	Eiweiss-N	773.2	431.8	587.4	593.7	591.9	475.3	676.7
	Lösl.-N	1200.2	196.1	357.6	515.4	639.6	566.2	597.6
	Ammon-N	44.2	82.7	200.9	266.8	367.0	289.3	328.3
	Amid-N	110.5	80.7	95.8	124.4	135.3	149.5	167.9
	Amino-N	1045.6	32.7	60.9	124.2	137.3	127.4	101.4
Amid/Ammon	2.5	0.97	0.47	0.46	0.36	0.51	0.51	0.71

unteren und den oberen Teilen reicher als der lösliche Stickstoff.

Der prozentuale Gehalt an den verschiedenen Fraktionen löslichen Stickstoffs im Verhältnis zum Gesamt-Stickstoff ist in Fig. 11 graphisch

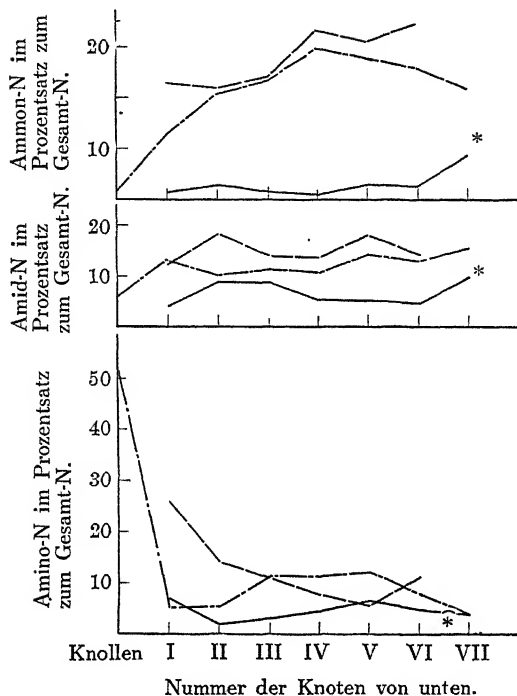


Fig. 11. Lösl.-N-Gehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. IV. 70tägige Züchtung im Gewächshaus.

— Blattspreite.

--- Blattstiel.

..... Stengel einschliesslich der betreffenden Knoten.

\* Blattspreite einschliesslich des betreffenden Blattstiels.

dargestellt. Wie man sieht, ist der Ammon-Stickstoffgehalt bei der Blattspreite sehr gering und unterliegt, mit einer Ausnahme beim 7ten Blatt, keinen nennenswerten Schwankungen. Der Gehalt an Amid-Stickstoff ist grösser als der an Ammon-Stickstoff und läuft mit dem des Ammon-Stickstoffs parallel. Bemerkenswert ist, dass beim Blattstiel der Ammon-Stickstoff, im Gegensatz zum vorigen Individuum, unter drei Fraktionen löslichen Stickstoffs am grössten und von der Basis nach der Spitze hin allmählich zunimmt und endlich 35% des Gesamt-Stickstoffs erreicht. Weiter ist bei ihnen der Gehalt an Amid-Stickstoff grösser als der an

Amino-Stickstoff, sodass seine Lage im Gegensatz zu der beim vorigen Individuum mittelständig ist.

In bezug auf den Gehalt an Ammon- und Amid-Stickstoff im Stengel, einschliesslich der betreffenden Knoten, liegt es ähnlich wie bei den Blattstielen.

Im Knollen ist wenig Ammon- und Amid-Stickstoff enthalten. Amino-Stickstoff dagegen wird im Knollen am meisten und zwar 52.98% des Gesamt-Stickstoffs gefunden.

Beim erster Knoten sinkt er auf 5% und steigt dann allmählich, aber nach der Spitze zu sinkt er wieder.

Die prozentuale Beziehung der einzelnen Fraktionen löslichen Stickstoffs, namentlich des Ammon-, Amid- und Amino-Stickstoffs, zu ihrer Gesamtheit ist in Tabelle 9 zusammengestellt. Daraus ist ersichtlich, dass sich in den Blattspreiten, wo reichlich Säure nachgewiesen worden ist, weniger Ammon-Stickstoff und reichlicher Amid-Stickstoff findet. Dagegen bildet der Ammon-Stickstoff bei den Blattstielen und dem Stengel einschliesslich der betreffenden Knoten den Hauptbestandteil des löslichen Stickstoffs, und allmählich steigt er von der Basis nach der Spitze zu, während der Amid-Stickstoff keine nennenswerten Schwankungen zeigt. Er ist in den Knollen am wenigsten nachweisbar. Der Gehalt an Amid-Stickstoff bleibt bei den Blattstielen ebenso wie bei dem Stengel niedriger als der an Ammon-Stickstoff. Der Amino-Stickstoff nimmt bei den Blattstielen von der Basis nach der Spitze hin ab. Er ist in den Knollen reichlich nachweisbar und beträgt 87% löslichen Stickstoffs. Der Gehalt an Amino-Stickstoff im Stengel wird wenig von den Schwankungen betroffen.

Was den Gehalt des Gesamt-Stickstoffs bei 1000 g Frischgewicht Material anbetrifft, so ist er im allgemeinen bei den Blattspreiten am grössten, bei Stengel einschliesslich der Knoten mässig und bei den Blattstielen am kleinsten. Der Gesamt-Stickstoff neigt, wie aus Tabelle 9 zu entnehmen ist, dazu, von der Basis nach der Spitze hin zuzunehmen. Dieselbe Neigung kann auch beim Gehalt des Eiweiss-Stickstoffs im Verhältnis zum Frischgewicht des Materials wahrgenommen werden.

Der Gehalt an löslichem Stickstoff bei 1000 g Frischgewicht Material verhält sich ganz ähnlich wie beim Individuum, Nr. 12. Und zwar ist er beim Blattstiel und Stengel einschliesslich der betreffenden Knoten grösser als bei der Blattspreite. Bei den Knollen ist er am grössten.

Die Reihenfolge der Menge des absoluten Ammon-Stickstoff, auf 1000 g Frischgewicht des Materials bezogen: Blattstiel > Stengel, einschliesslich der betreffenden Knoten > Blattspreite.

Die Reihenfolge der Menge des absoluten Amid-Stickstoffs, auf 1000 g Frischgewicht des Materials bezogen: Stengel, einschliesslich der betreffenden Knoten > Blattstiel > Blattspreite.

Und die Reihenfolge der Menge des Amino-Stickstoffs: Knollen > Blattstiel > Blattspreite > Stengel, einschliesslich der Knoten.

Bei den Knollen macht der Amino-Stickstoff den Hauptteil des Gesamt-Stickstoffs aus. Der Quotient Amid/Ammon ist beim Blattstiel am kleinsten und beim Stengel ein mittlerer. Jedenfalls ist das Überwiegen des Ammoniaks gegenüber dem Amid augenfällig. Dagegen bei den Knollen und Blattspreiten liegt es grade umgekehrt, und das Amid überwiegt das Ammoniak stark.

### Versuch 3.

Zum selben Zweck wurde weiter ein anderes Individuum (Nr. 6), das 124 Tage gezüchtet war und schon ausgeblüht hatte, analysiert. Die Ergebnisse der Säurebestimmung sind in üblicher Weise in Tabelle 10 zusammengestellt. Wie man aus nebenstehender Figur (Fig. 12) ersieht,

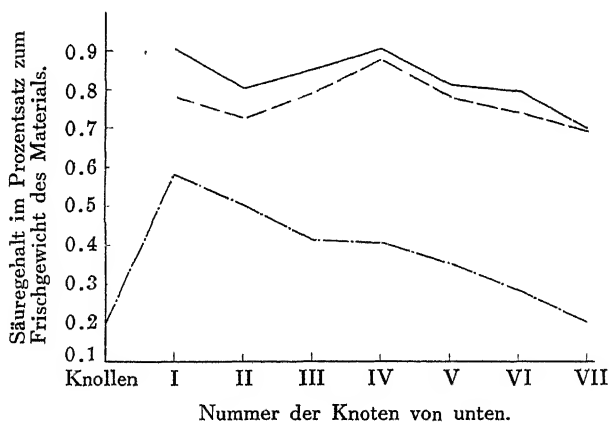


Fig. 12. Der Säuregehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. 6. 124tägige Züchtung im Gewächshaus.

———— Blattstiel.      - - - - - Blattspreite.  
 - · - · - Stengel einschliesslich der betreffenden Knoten.

ist der Säuregehalt im allgemeinen viel grösser als beim vorigen Individuum, und die Kurve verläuft meistens sehr glatt. Der Säuregehalt des Stengels einschliesslich der betreffenden Knoten nimmt aber gerade in umgekehrter Richtung von der Basis nach der Spitze hin ab, und dies stimmt mit

TABELLE 10.

Der Säure- und Stickstoffgehalt in den verschiedenen Pflanzenteilen  
von *Begonia Evansiana* ANDR. Nr. 6. 124tägige  
Züchtung im Gewächshaus.

(a) Blattspreite.

Nr. d. Blattspreite von unten		I	II	III	IV	V	VI	VII
Frischgewicht d. Materials mg		229.790	291.249	344.595	308.526	257.082	262.311	144.999
Oxalsäure mg		1.799	2.114	2.729	2.698	1.995	1.950	1.013
Prozent		0.78	0.72	0.79	0.87	0.77	0.74	0.69
mg Oxalsäure in 1000 g Frischgew.		7828.8	7258.3	7919.4	8744.8	7760.1	7433.9	6986.2
Frischgewicht d. Materials mg		745.989	916.646	1163.441	1046.979	848.185	849.866	512.116
Gesamt-N mg		1.8214	1.3356	2.5374	2.6600	2.5018	2.5984	1.7191
Gesamt-N in % d. Frischgewichtes		0.24	0.14	0.21	0.25	0.29	0.30	0.33
N in % d. Gesamt-N	Eiweiss-N	91.24	88.05	92.05	88.95	90.77	86.75	86.81
	Lösl.-N	8.76	11.95	7.95	11.05	9.23	13.25	13.19
	Ammon-N	2.42	3.30	1.57	2.21	1.93	2.17	2.93
	Amid-N	1.83	4.40	4.96	4.73	7.27	4.04	6.59
	Amino-N	4.49	4.24	1.40	6.57	2.93	7.03	3.66
N in % d. Lösl.-N	Ammon-N	27.63	27.63	19.79	20.00	20.91	16.41	22.22
	Amid-N	21.05	36.84	62.50	42.86	47.27	30.48	50.00
	Amino-N	51.32	35.53	17.71	37.14	31.82	53.11	27.78
mg N in 1000 g Frischgewicht	Gesamt-N	2441.6	1457.0	2180.9	2541.7	2949.6	3057.4	3357.1
	Eiweiss-N	2227.7	1282.9	2007.7	2261.9	2677.3	2652.2	2914.2
	Lösl.-N	213.9	174.1	173.2	280.8	272.3	405.2	442.9
	Ammon-N	59.1	48.1	34.3	56.2	56.9	66.5	98.4
	Amid-N	45.0	64.1	108.3	120.3	128.7	123.5	221.5
	Amino-N	109.8	61.9	30.6	104.3	86.7	215.2	123.0
Amid/Ammon		0.76	1.33	3.15	2.14	2.26	1.85	2.25

## (b) Blattstiel.

Nr. d. Blattstiels von unten		I	II	III	IV	V	VI	VII
Frischgewicht d. Materials mg		171.734	232.184	337.455	395.790	232.527	172.466	86.588
Oxalsäure mg		1.551	1.873	2.844	3.567	1.889	1.379	0.610
Prozent		0.90	0.80	0.84	0.90	0.81	0.79	0.70
mg Oxalsäure in 1000 g Frischgew.		9031.4	8066.8	8427.7	9012.3	8123.7	7995.7	7044.8
Frischgewicht d. Materials mg		453.462	494.852	1053.285	634.519	640.009	762.538	532.618
Gesamt-N mg		0.3968	0.4298	0.7178	0.4256	0.4970	0.6832	0.4970
Gesamt-N in % d. Frischgewichtes		0.08	0.08	0.06	0.06	0.07	0.08	0.09
N in % d. Gesamt-N	Eiweiss-N	75.65	61.03	53.02	64.80	61.97	60.28	53.81
	Lösl.-N	24.35	33.97	46.98	35.19	38.03	39.72	46.19
	Ammon-N	11.64	21.00	23.40	13.81	14.36	15.16	12.67
	Amid-N	5.29	6.84	9.94	7.89	8.45	9.22	8.45
	Amino-N	7.40	12.12	13.62	13.55	15.21	15.36	25.07
N in % d. Lösl.-N	Ammon-N	47.83	53.91	49.82	39.25	37.78	33.14	27.44
	Amid-N	21.74	17.55	21.17	22.43	22.22	23.20	18.29
	Amino-N	30.43	28.54	29.01	31.32	40.00	38.66	54.27
mg N in 1000 g Frischgewicht	Gesamt-N	875.0	868.5	682.5	670.7	776.6	895.9	933.1
	Eiweiss-N	662.0	530.0	361.3	434.7	481.3	539.8	502.0
	Lösl.-N	213.0	338.5	320.2	236.0	295.3	356.1	431.1
	Ammon-N	101.9	182.5	159.5	92.7	111.6	135.9	118.3
	Amid-N	46.3	59.4	67.8	52.9	65.6	82.5	78.9
	Amino-N	64.8	96.6	92.9	90.4	118.1	137.7	233.9
Amid/Ammon		0.45	0.32	0.42	0.57	0.58	0.60	0.66



(c) Knollen und Stengel einschliesslich der betreffenden Knoten.

Pflanzenteil		Knollen	I Stengel	II Stengel	III Stengel	IV Stengel	V Stengel	VI Stengel	VII Stengel
Frischgewicht d. Materials mg		264.774	326.469	187.562	125.676	181.750	241.959	259.549	145.941
Oxalsäure mg		0.324	1.895	0.941	0.519	0.749	0.856	0.741	0.294
Prozent		0.12	0.58	0.50	0.41	0.41	0.35	0.28	0.20
mg Oxalsäure in 1000 g Frischgew.		1223.6	5804.5	5017.0	4129.6	4121.0	3537.7	2854.9	2014.5
Frischgewicht d. Materials mg		1341.195	772.016	945.688	1036.325	957.152	966.525	998.011	662.774
Gesamt-N mg		4.6648	1.2642	10388	1.2110	1.3514	1.7402	1.9122	1.2306
Gesamt-N in % d. Frischgew.		0.35	0.16	0.11	0.12	0.14	0.18	0.19	0.19
N in % d. Gesamt-N	Eiweiss-N	26.17	39.87	67.65	56.94	53.38	46.18	47.07	46.07
	Lösl.-N	73.83	60.13	32.35	43.06	46.62	53.82	52.93	53.93
	Ammon-N	0.32	3.49	11.93	20.12	18.34	11.10	12.52	10.07
	Amid-N	7.38	6.65	10.51	10.75	10.88	9.17	7.69	12.29
	Amino-N	66.13	50.00	9.91	12.14	17.40	33.23	32.73	31.57
N in % d. Lösl.-N	Ammon-N	0.43	5.80	36.88	46.77	39.33	20.63	23.65	18.67
	Amid-N	10.00	11.05	32.50	25.00	23.33	17.04	14.53	22.78
	Amino-N	89.57	83.15	30.62	28.23	37.33	62.33	61.82	58.55
mg N in 1000 g Frischgewicht	Gesamt-N	3478.1	1637.5	1098.5	1168.6	1411.9	1800.5	1916.2	1856.7
	Eiweiss-N	910.2	652.8	743.2	666.0	753.7	831.5	902.0	855.5
	Lösl.-N	2567.9	984.7	355.3	502.6	658.2	969.0	1014.2	1001.2
	Ammon-N	11.0	57.1	131.0	235.1	258.9	199.9	239.9	186.9
	Amid-N	256.8	108.8	115.5	125.6	153.6	165.1	147.3	228.1
	Amino-N	2300.1	818.8	108.8	141.9	245.7	604.0	627.0	586.2
Amid/Ammon		23.34	1.90	0.88	0.53	0.59	0.82	0.61	1.22

meiner ersten Mitteilung gut überein. Daraus kann man schliessen, dass der Säuregehalt, je nach den verschiedenen Entwicklungsstadien, ganz verschieden ausfällt, und dass beim Wachstumsaufhören infolge der umgekehrten Stoffwechselvorgänge sogar die umgekehrte Reihenfolge des Säuregehalts bestehen kann. Bei unserer Pflanze nimmt die Säuremenge mit dem Alter zu.

Die Ergebnisse der Stickstoffbestimmungen sind in den vorstehenden Tabellen 10 zusammengestellt. Im grossen und ganzen verhält sich auch hier der Gehalt an Eiweiss- und löslichem Stickstoff zum Gesamt-Stickstoff ähnlich wie beim vorigen Individuum. Nur darin liegt eine Abweichung, dass beim Blattstiel das Mengenverhältnis des Eiweiss- und des löslichen Stickstoffs umgekehrt war. Die Menge des löslichen Stickstoffs in den Knollen ist viel grösser als die des Eiweiss-Stickstoffs.

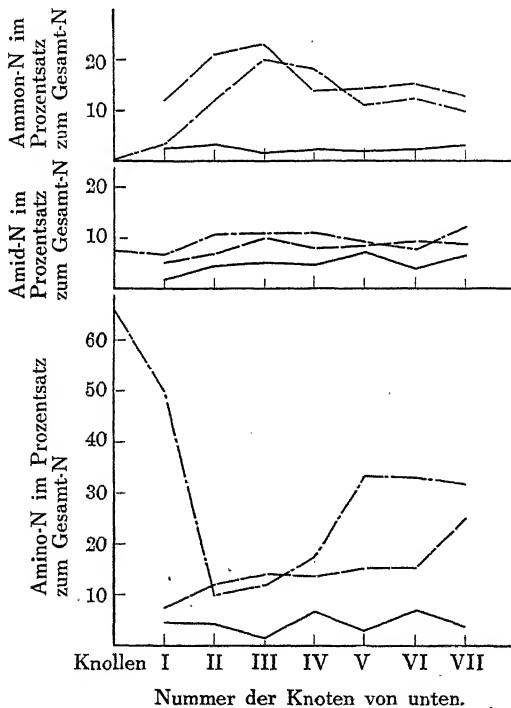


Fig. 13. Lösl.-N-Gehalt in verschiedenen Pflanzenteilen von *Begonia Evansiana* ANDR. Nr. 6. 124tägige Züchtung im Gewächshaus.

Was die einzelnen Fraktionen löslichen Stickstoffs im Verhältnis zum Gesamt-Stickstoff betrifft, so kann man zunächst aus Fig. 13 ersehen, dass sich bei diesem Individuum meistens bedeutend mehr Amino-Stickstoff als andere Fraktionen findet. Dagegen ist der Gehalt an Ammon- und Amid-Stickstoff in allen Körperteilen stark zurückgegangen. Nur ist das für die vorigen Fälle geltende Verhältnis insofern bestehen geblieben, als beim Blattstiel und beim Stengel einschliesslich der betreffenden Knoten der Ammon-Stickstoff grösser als der Amid-Stickstoff ist und bei den Blattspreiten dieses Verhältnis gerade umgekehrt liegt.

Weiter ist aus dem prozentualen Gehalt der einzelnen Fraktionen des löslichen Stickstoffs zu ihrer Gesamtheit ersichtlich, dass bei den Blattspreiten der Ammon-Stickstoff beinahe einen beständigen Wert und der Amid- und der Amino-Stickstoff einen höheren Wert zeigen. Beim Blattstiel dagegen macht der Ammon-Stickstoff den grössten Teil des löslichen Stickstoffs aus und sinkt von der Basis nach der Spitze hin. In den Blattstielen ist verhältnismässig wenig Amid-Stickstoff nachweisbar, und er läuft parallel zur Abzisse, während der Amino-Stickstoffgehalt, im Gegensatz zum Ammon-Stickstoff, von der Basis nach der Spitze hin ansteigt. In bezug auf den Ammon- und den Amid-Stickstoff im Stengel einschliesslich der betreffenden Knoten ist eine ähnliche Neigung wie bei den Blattstielen wahrzunehmen.

In den Knollen aber findet sich der meiste Amino-Stickstoff. Beim Stengel sinkt er am 3. Knoten zum Minimum und steigt allmählich wieder nach der Spitze hin.

Was schliesslich den Gehalt des Gesamt- und des Eiweiss-Stickstoffs bei 1000 g Frischgewicht Material betrifft, so kann man sehen, dass sie in den Blattspreiten am grössten und im Blattstiel am kleinsten sind. Die Reihenfolge der Menge des löslichen Stickstoffs und der einer Fraktion, des Ammon-Stickstoffs, bei 1000 g Frischgewicht Material: Stengel, einschliesslich der betreffenden Knoten > Blattstiel > Blattspreiten. Bei der der Amid- und Amino-Stickstoffmenge: Stengel, einschliesslich der betreffenden Knoten > Blattspreiten > Blattstiel. Der Quotient Amid/Ammon ist beim Blattstiel und Stengel einschliesslich der betreffenden Knoten klein und bei der Blattspreite gross.

Aus obigen Ergebnissen darf man vor allem schliessen, dass in den Blattstielen und dem Stengel, einschliesslich der betreffenden Knoten, der Zunahme des Säuregehalts der grössere Gehalt an Ammon- bzw. Amid-Stickstoff beinahe entspricht. Dies scheint dafür zu sprechen, dass die Säure bei der Desaminierung der Aminosäuren entsteht. In den Blatt-

spreiten, in denen viel mehr Säure als im Stengel nachweisbar ist, war die Ammon-Stickstoffmenge in allen untersuchten Fällen immer am kleinsten. Weiter ist für sie charakteristisch, dass, im Gegensatz zum Blattstiel bezw. Stengel, die Menge des Ammon-Stickstoffs sehr viel kleiner als die des Amid-Stickstoffs war, sodass das Verhältnis Amid/Ammon immer grösser als 1 ausfiel. Allerdings war dieses Verhältnis in den verschiedenen Entwicklungsstadien verschieden und zwar bei einem jüngeren Individuum für alle Pflanzenteile ausnahmslos grösser als 1. Dies widerspricht aber dem obigen Ergebnis insofern nicht, als die Grösse dieser Verhältniszahlen bei den Blattspreiten die bei den Blattstielen und den Stengel stark überwiegt. Dieses unerwartete Tatsache ist meines Erachtens dahin zu deuten, dass das beim Eiweissabbau entstandene Ammoniak in den Blattspreiten schneller zur Eiweissynthese verwendet wurde, oder dass sich bei der Säurebildung noch ein anderer Vorgang als die Desaminierung der Aminosäure mitbeteiligt. Daraus entsteht die Frage, ob nicht in den Blattspreiten meiner Versuchspflanze neben der Oxalsäure noch andere Säuren gebildet werden. Es ist weiter noch unklar mit welchen Amiden hier für den Amid-Stickstoff zu rechnen ist. Um diese Frage zu klären, bedarf es noch weiterer Untersuchungen.

#### IV. ZUSAMMENFASSUNG.

Die Ergebnisse vorstehender Untersuchungen lassen sich, wie folgt, zusammenfassen.

1. Mit quantitativen mikrochemischen Methoden wurden der Stickstoff- und der Säurestoffwechsel von Blättern, Stengel, Knollen, Bulbillen und Blüten der *Begonia Evansiana* ANDR., unter besonderer Berücksichtigung ihrer gegenseitigen Beziehungen, untersucht.
2. Als die Verbrennung beschleunigendes Reagens erwies sich Überchlorsäure als geeigneter als Perhydrol.
3. In jeder Blattspreite nimmt die Säure in der Nacht zu, und die Zunahme ist in den oberen, jungen Blattspreiten grösser als in der unteren, alten desselben Individuums.
4. Die Ansäuerung nimmt bei allen Organen mit ihrem Alter zu.
5. Bei den Knollen und Bulbillen überwiegt der lösliche Stickstoff ausnahmslos den Eiweiss-Stickstoff. Der Beginn des Sprossens der Knospe aus den Knollen kennzeichnet sich dadurch, dass hier der Amino-Stickstoff eine durchaus beherrschende Form des löslichen Stickstoffs darstellt, und die Amide vollständig zurücktreten. Der Ammon-Stickstoff ist bei den

Knollen ebenso wie bei den Bulbillen ganz gering.

6. Bei der Blattspreite gibt es im allgemeinen eine Nachtzunahme des Ammon- und des Amid-Stickstoffs, während der Eiweiss-Stickstoff keine merkwürdigen Schwankungen zeigt.

7. Bei den Blüten entspricht der Ansäuerung eine überwiegende Menge des Ammon- bzw. Amid-Stickstoffs.

8. In dem Blattstiel und dem Stengel scheint ein Parallelismus zwischen dem Säuregehalt und dem Ammon- bzw. dem Amid-Stickstoffgehalt zu bestehen. Dort überwiegt die Menge des Ammon-Stickstoffs meistens die des Amid-Stickstoffs.

9. In den Blattspreiten findet sich der Stickstoff in Form von Amid viel reichlicher als in Form von Ammon, sodass der Säuregehalt nicht so gut dem Ammon-Stickstoffgehalt entspricht, wie man anfangs erwarten könnten, wenn überhaupt auch in den Blattspreiten aus dem Eiweisstoffwechsel Oxalsäure entstehen sollte. Ob dieses Ergebnis der stärkeren Eiweissynthese in den Blattspreiten zuzuschreiben ist oder durch einen anderen Vorgang als die Desaminierung der Aminosäure bedingt ist, muss noch dahingestellt bleiben.

Vorliegende Arbeit wurde im Jahre 1932 im Biologischen Institut der Kaiserlichen Tôhoku-Universität zu Sendai ausgeführt. Die Anregung dazu gab mein hochverehrter Lehrer, Herr Prof. Dr. Y. YAMAGUTI. Dafür und für die dauernde Förderung, die er meiner Untersuchung zuteil werden liess, sage ich ihm auch an dieser Stelle meinen aufrichtigsten Dank.

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THE RELATIVE VALUES OF CATIONS IN PROTECTING THE  
MEMBRANE FORMING CAPACITY OF THE EGGS OF  
THE ECHINOIDS, *CLYPEASTER JAPONICUS* AND  
*TEMNOPLEURUS HARDWICKII*.\*

By

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In a series of studies I have shown that when the unfertilized eggs of echinoderms are subjected for a short time to the action of a solution of non-electrolyte which is isosmotic with sea water and is neutral or alkaline, such eggs suffer an irreversible change in that they lose their power to form fertilization membranes<sup>1,2)</sup>. The effectiveness of the non-electrolyte solution in causing this change is related to the hydroxyl ion concentration of the solution and it is apparent from the work on the eggs of *Strongylocentrotus purpuratus* that the hydroxyl ion is responsible for the effect<sup>3)</sup>. Furthermore this destructive action of the hydroxyl ion with reference to the pre-membrane stuff is antagonized and may be completely inhibited by the cations of the alkali and alkaline earth series when they are added in the form of chlorides to the solution of non-electrolyte. In the case of *Strongylocentrotus purpuratus* the divalent ion  $\text{Ca}^{++}$  proved approximately 100 times as effective as the monovalent  $\text{Na}^+$ , and in the case of *Echinus microtuberculatus* and *Paracentrotus lividus* the coefficient was about 60<sup>4)</sup>. These observations led me to suggest that the protective effect of the cations is chiefly a function of the valence and attention was called to the fact that similar relations had been established by LUCKÉ and McCUTCHEON<sup>5)</sup> in their studies on the permeability to water of the unfertilized eggs of *Arbacia*. At the same time the evidence did not allow the assumption that the two phenomena depend on the same variable. In this connection I pointed out that the two reactions probably are quite different since permeability effects are reversible while the loss of membrane forming capacity is irreversible.

LUCKÉ and McCUTCHEON were able further to explore the effects of

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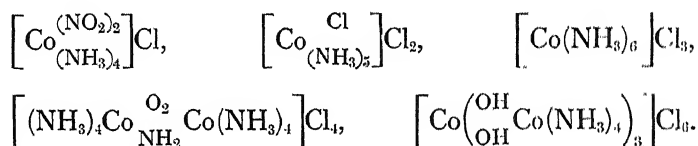
\*) Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 102.

\*\*) Visiting Professor in Biology on the Rockefeller Foundation.



valence on permeability to water by means of a series of cobaltamine chlorides in which the cations possessed valences up to 6. In general their results showed that the effectiveness of the cations in diminishing permeability increased with the valence, although the increment was not found to be uniform with each added valence. My own experiments have previously been limited to the effects of monovalent and divalent cations, for the reason that those of higher valence such as  $\text{Al}^{+++}$  affect the pH of the solution. I have been enabled, however, to work with the higher valence cobaltamines through the kindness of Dr. LUCKÉ who very generously gave me a stock of cobaltamine chlorides which had been prepared by Professor THOMAS B. McCUTCHEON of the University of Pennsylvania. To both Dr. LUCKÉ and Dr. McCUTCHEON I take pleasure in expressing my hearty thanks for this very substantial gift.

The formulae of the 5 cobaltamine chlorides used are as follows:



During the summer of this year I have been able to study the question using as material the eggs of two echinoids, *Clypeaster japonicus* at Misaki and *Temnopleurus hardwickii* at Asamushi. In making an individual experiment, 25 cc. of solution was used which was made up of a M solution of urea containing the desired salt in known quantity, and the whole brought to pH 8. The solution was put into a petri dish, a drop of a thick suspension of the eggs added and the whole thoroughly stirred. The practice of taking the eggs directly from sea water introduced a constant error of approximately 3 per cent. At the end of about  $2\frac{1}{2}$  minutes the eggs were gathered together by gentle rotation of the dish and at exactly 3 minutes as many as possible were removed from the dish with 2 or 3 drops, by means of a pipette, and put into a dish of sea water. Sperm was added and 3 to 5 minutes afterward the per cent of membranes formed was estimated, with the object of determining the amount of a given salt necessary to preserve the membrane forming capacity of approximately 50 per cent of the eggs. For example, in one experiment,  $\text{BaCl}_2$  in concentration 0.0045 M preserved 10 per cent of the membranes and 0.006 M preserved 80 per cent, then the value which was taken is the approximate mean, namely, 0.005 M. A series was rejected if, before the end of the experiments, the eggs of that particular

lot failed to form membranes after exposure to a solution of unquestionably protective action. The table shows the average values of three completed series of experiments in the case of *Temnopleurus*, and of two series for *Clypeaster*, except that the values for RbCl and CsCl are from a single series and the cobaltamine chloride values are from one series for each form.

TABLE.

*Each number denotes the molecular concentration of the corresponding salt, given in the left hand column, which is just sufficient in a solution of urea M/1 at pH 8, to protect the membrane forming capacity of 50 per cent of the eggs.*

	<i>Clypeaster</i>	<i>Temnopleurus</i>
LiCl		0.213
NaCl	0.250	0.213
KCl		0.276
RbCl		0.220
CsCl		0.250
MgCl <sub>2</sub>	0.0074	0.0068
CaCl <sub>2</sub>	0.0060	0.0042
SrCl <sub>2</sub>	0.0064	0.0068
BaCl <sub>2</sub>	0.0042	0.0046
1-valent cobaltamine Cl	—	—
2-valent    "    "	0.0025	0.002
3-valent    "    "	0.00025	0.00004
4-valent    "    "	0.00020	—
6-valent    "    "	0.00005	—

As in previous experiments, the ions of each valence series are of the same order of effectiveness within their own group. Leaving aside for the time being, the individual differences within each series let us compare the effective concentrations. The average concentration of divalent ions for *Clypeaster* is 0.0060 M, while that of Na<sup>+</sup> is 0.250 M. The divalent ion is therefore 42 times as effective as the monovalent Na<sup>+</sup>. In *Temnopleurus* the divalent average effective concentration is 0.0056 M, the monovalent 0.234 M, yielding a coefficient of 42. This exact identity of coefficients is of course accidental but we can be sure the valence coefficients in question lie close to 50. These figures demonstrate the fact which I have emphasized before, namely, that the factor of greatest magnitude in the action of the metal ions is valence. Within each periodic group there

are relatively minor differences characteristic of the individual ion, differences which are superposed upon the valence effect. Thus  $Mg^{++}$  is always less effective than  $Ca^{++}$ , while  $Ba^{++}$  equals or exceeds  $Ca^{++}$  in effectiveness. It is apparent from these results that the fashion of expressing comparative ion effects as a series which includes both valence groups may be entirely misleading. Thus the expression  $Ca^{++} > Mg^{++}$  for effectiveness in the experiment with *Temnopleurus* means that  $1\ Ca^{++} = 1.52\ Mg^{++}$ , but to write another relation which is also true, namely,  $Ca^{++} > Na^{+}$  means something totally different because here  $1\ Ca^{++} = 51\ Na^{+}$ ! The quantitative fallacy involved in writing  $Ca^{++} > Mg^{++} > Na^{+}$  to express this set of facts is apparent. This example will suffice to show the impossibility of representing comparative ion effects by means of a series which includes ions of differing valences.

The experiments with the cobaltamines were successful throughout the group with the eggs of *Clypeaster*, but with *Temnopleurus* the 4- and 6-valent salts cytolized the eggs so that only the 2- and 3-valent salts gave reliable results. In no case have I been able to obtain protection with the 1-valent salt, probably for the reason that it is not possible to make a solution of sufficient concentration. The 2-valent cobaltamine is the most constant of them all in its effectiveness, giving with all eggs tried (including *Arbacia punctulata* and *Strongylocentrotus pulcherrimus*) an almost identical effective concentration, a concentration number which is one-half to one-third that of the divalent metal ions, a difference which may be due to the high molecular weight of the cobaltamine salt. The valence interval 2 to 3 gives a coefficient of 10 for *Clypeaster*, 50 for *Temnopleurus*. But for *Clypeaster* the 4-valent ion is only slightly more effective than the 3-valent one. In *Clypeaster* the 6-valent ion is 4 times as effective as the 4-valent. It is therefore evident that an additional valence may mean very different things quantitatively depending upon the number of valences present. These irregularities may be due to differences in the relations of the valences within the ion, or to the configuration of the particular ion concerned. At present our information does not permit a decision.

#### SUMMARY.

Use was made of the general property of the unfertilized eggs of echinoderms by which in a solution of non-electrolyte they lose irreversibly their power to form fertilization membranes. The eggs of *Clypeaster japonicus* and of *Temnopleurus hardwickii* were used. Comparison of the

effective concentrations of the salts showed that the divalent alkaline earth metals are approximately 42 times as effective as the monovalent alkali metal ions.  $\text{Ca}^{++}$  proved to be consistently slightly more effective than  $\text{Mg}^{++}$ . The series of cobaltamine chlorides as used by LUCKÉ and McCUTCHEON gave no protection in the case of the 1-valent salt. Beginning with the 2-valent cobaltamine there was found to be an increasing effect by irregular steps with each added valence. The highest coefficient was found between the 2- and 3-valent, the least between the 3- and 4-valent salts.

It gives me pleasure to express my thanks to Mr. YOSHII and Mr. ERI of the Misaki Station; and to Mr. KOKUBO and Mr. TAMURA of the Asamushi Station for many courtesies extended to me. I am also indebted to Mr. FUKUDA at Misaki and to Mr. KOBAYASHI at Asamushi for determining the chlorine in my solutions.

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ON THE PRESENCE OF THE IMMOVABLE CORTICAL  
CYTOPLASM IN THE CENTRIFUGED SEA-URCHIN  
EGG AND ITS IMPORTANCE ON THE DETER-  
MINATION OF THE POLARITY.  
(PRELIMINARY REPORT).

By

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In many species the location of the pole of the egg is unaffected by the redistribution of the visible materials in the egg cytoplasm by weak centrifuging. To analyse the causality of this problem, I carried out the centrifuging experiment with the egg of sea-urchins in the summer of 1933 at Misaki.

The unfertilized egg of *Heliocidaris crassispina* (A. AGASSIZ) was centrifuged thirty minutes at a speed of three thousand revolutions per minute, which is about 1400 times gravity. The egg showed a sharp stratification of three layers. The centripetal layer is a small amount of opaque granules. The centrifugal layer is also a compact and opaque cytoplasm, which is about half the volume of the egg. The middle layer is a clear cytoplasm. The nucleus of the egg is carried into the clear protoplasmic zone, and lies just beneath the centripetal opaque layer. In some cases the eggs were elongated in the direction centrifuging, but they were never torn to fragments. The eggs were easily fertilized. In the course of segmentation I could trace the three layers till the eight-cell stage in the living egg. The centripetal granular layer was detected in the dark field illumination even in the gastrula. But the position of the granular layer had no connection with the axis of the gastrula, not with the micromeres of the sixteen-cell stage. The eggs became normal plutei.

The material was fixed and observed in sections. The centripetal zone is dissolved away by the treatment of paraffin section, and does not take stains. The centrifugal layer is eosinophilous. The middle clear zone is a basophil chromatic material, and is stained intensively with HEIDENHAIN'S hematoxylin. In the course of cleavage the basophilous middle layer and the eosinophilous centrifugal layer mingle with each other, but it was

possible to recognize the direction of centrifuging by the difference of the intensity of the staining in the blastomeres.

The results of my experiments on *Heliocidaris* agree with those on *Arbacia*. The direction of centrifuging did not affect the original location of the axis of the egg. For the interpretation of this result we must demonstrate the substance in the egg which is immovable by centrifuging. In the first place I put my attention on the cortical cytoplasm. The cortical cytoplasm of the normal egg of *Heliocidaris* contains basophilous granules. And by centrifuging at the speed mentioned above the granule of this portion of the unfertilized egg did not show shifting at all. On the contrary the basophilous granules of the inner part accumulate in the middle layer. At the centripetal end, where the opaque granules accumulate, I observed a thin layer of basophilous cytoplasm. The structure of this portion is identical with that of the other cortical side of the egg. This shows that the cortical cytoplasm is so rigid that it is not destroyed by the centripetal pressure of the opaque lipoid granules. At the centrifugal side there is also cortical cytoplasm of the same structure. In short the cortical cytoplasm is not affected by centrifuging and retains the condition of the normal egg.

This fact is also observed in the centrifuged unfertilized egg of *Temnopleurus toreumaticus* (KLEIN). In this species the cortical cytoplasm contains much more basophilous granules than in *Heliocidaris*, and for this reason it shows a sharp contrast between the cortical cytoplasm and inner cytoplasm. The nucleus of the egg of *Temnopleurus* lies at the centrifugal end, or in the other words the nucleus has the large specific gravity. In some cases the centrifugal end of the egg juts out by the centrifugal pressure of the nucleus, but even in this case the cortical cytoplasm remains undestroyed. In *Clypeaster japonicus* DÖDERLEIN and *Astriclypeus manni* VERRILL there are less basophilous granules in the cortical cytoplasm. But in the centrifuged egg at the side of the basophilous zone there is also a sharp contrast between basophilous inner portion and unstained cortical portion.

It is well known that in the course of normal development the inner cytoplasm is stirred up by the protoplasma-streaming and the motion of the nucleus. Therefore the direction of the pole of the molecules of the inner protoplasm must be unstable even in the normal egg. It is illogical to suppose a structure or substance which retains the polarity of the egg analogous to a crystal in the inner part of the egg cytoplasm. And the hypothesis of the axial gradient should not be adapted in respect with

the inner cytoplasm, because the physiological axial gradient ought to be based upon the qualitative or quantitative nature of the distribution of materials. And this must be immovable by the slow centrifuging. The cortical cytoplasm is only the substance in which immovability can be demonstrated. Therefore the structure, on which the polarity of the egg depends, must be delineated in the cortical cytoplasm. If the centrifuging is strong enough to destroy the structure of the cortical cytoplasm it may be possible to alter the polarity of the egg, in which case the gradient of the inner cytoplasm may affect the polarity. The discrepancies of the results obtained by RUNNSTRÖM, LINDAHL and myself were caused by the difference of relative speed of centrifuging against the rigidity of the egg cytoplasm.

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# MITOSEN IM ANTHERIDIUM VON *SARGASSUM* *CONFUSUM* AG.<sup>1)</sup>

VON

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(Mit Tafeln IX-X)

(Eingegangen am 18. Oktober, 1933)

Unter den Fucaceen wurde die Gattung *Sargassum* in letzter Zeit von verschiedenen Autoren zytologisch untersucht. Über die Karyokinese im Oogonium von *Sargassum enerve* veröffentlichten TAHARA und SHIMOTOMAI (1926) eine kurze Mitteilung. KUNIEDA (1928) studierte die Kernteilung und die Befruchtung bei *Sargassum Horneri*, und OKABE (1929, 1930) untersuchte die Meiosis im Oogonium und die Mitosen im keimenden Embryo derselben Spezies. Aber die Mitosen im Antheridium dieser Gattung sind bis jetzt noch von niemandem genauer untersucht worden. In diesem Frühling studierte ich sie während meines zweimonatigen Aufenthalts in der biologischen Station der hiesigen Universität in Asamushi unter Leitung Herrn Prof. Dr. M. TAHARAS, dem ich für seine vielseitigen Ratschläge zu grossem Dank verpflichtet bin.

Als Material gebrauchte ich *Sargassum confusum*, das in dieser Gegend sehr üppig wuchert. Beim *Sargassum* beginnt die Karyokinese des Antheridiumkerns zugleich mit der des Oogoniumkerns. Die verschiedenen karyokinetischen Phasen lassen sich selbst beim lebenden Material mit Essigkarmin klar erkennen. Darum ist es leicht, das Material in den geeigneten Phasen zu fixieren. Da die Antheridien dieser Alga mit Schleimhaut bekleidet sind, muss man auf die Fixierung besonders achten. Durch wiederholte Versuche hat sich folgende Methode als die beste erwiesen. Zuerst bereitet man zwei Lösungen. Die erste Lösung besteht aus zwei Vorratslösungen a) und b); die zwei müssen kurz vor Gebrauch zu gleichen Teilen gemischt werden.

- a) Gesättigte Lösung von Pikrinsäure ..... 50 ccm,  
Eisessigsäure ..... 5 ccm,  
Chromsäure ..... 1 g.
- b) Gesättigte Lösung von Pikrinsäure ..... 25 ccm,

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<sup>1)</sup> Contributions from the Marine Biological Station, Asamushi, Aomori-Ken, No. 103.

40%iges Formalin ..... 25 ccm,  
 Harnstoff ..... 0.5 g.

Die zweite Lösung ist die, die ich bei meiner Untersuchung (1932) der Befruchtung von *Coccophora* benutzt habe.

Das vorliegende Material wurde mit dieser gemischten Flüssigkeit meistens 4–5 Stunden lang fixiert. Um mit diesem Fixierungsmittel gute Ergebnisse zu erzielen, ist es wesentlich, die Rezeptakel so klein wie möglich zu zerschneiden. Die  $7\mu$  dick geschnittenen Paraffinschnitte werden mit HEIDENHAIN'S Eisenalaunhämatoxylin gefärbt.

Fig. 1 zeigt den sich im vollständigen Ruhestadium befindlichen Antheridiumkern. In Fig. 2 und 3 sieht man ein früheres Synapsisstadium, während dessen ein kleines, synaptisches Knäuel an einer Seite des Kerns vorragt. Häufig bemerkte ich zwei solche synaptische Knäuel sich gegenüber an beiden Seiten des Kerns, wie Fig. 4 zeigt. Auch YAMANOUCHI (1909) schreibt von dieser Erscheinung im Antheridium von *Fucus*. Fig. 5 stellt ein noch späteres Synapsisstadium dar. Im fortschreitenden Stadium strecken sich die Fäden des synaptischen Knäuels in der Kernhöhle. Darauf folgt das Spiremstadium (Fig. 6). Dabei kann man einen fortlaufenden Spiremfaden mit stark färbbaren Knorren sehen. Diese Knorren treten allmählich deutlich hervor, und dann folgt das Diakinesestadium (Fig. 7). Die Bivalentchromosomen zeigen meistens die Y—, X—, O—, und II—Form, wie das TAHARA (1929) bei der Ovogenese von *Coccophora* beschrieben hat (Fig. 8). Fig. 9 zeigt ein späteres Diakinesestadium. In diesem Stadium ist der Nukleolus schon verschwunden. Wie aus Fig. 3 und 6 zu ersehen, gibt es zuweilen ein Körperchen in der Nähe des Nukleolus. Etwas Ähnliches wurde von WILLIAMS (1906) im Kern der Tetrasporenmutterzelle von *Dictyota* und von CARTER (1927) bei *Padina* bemerkt und als „chromophilous spherule“ bezeichnet. Auch TAHARA (1929) hat dies im Oogoniumkern von *Coccophora* und OKABE (1929) in dem von *Sargassum Horneri* gefunden. Nach Auflösung der Kernmembran erfolgt vollständige Metaphase, wobei sich die 32 Chromosomen ohne Schwierigkeit zählen lassen (Fig. 10, 11). Obwohl YAMANOUCHI (1909) bei der Meiosis im Antheridium von *Fucus* Zentrosomen bemerkt hat, konnte ich sie bei meiner Pflanze in keinem karyokinetischen Stadium beobachten. Die Anaphase geht regelmässig vor sich (Fig. 12). Die reduzierten Chromosomen wandern nach beiden Polen. In der Telophase berühren sich die beiden Tochterkerne eng (Fig. 13). Nach kurzer Pause beginnt die homöotype Teilung (Fig. 14). Nach dieser Teilung entstehen natürlich vier Kerne (Fig. 15), die dann noch vier weitere Teilungen ausführen

(Fig. 16, 17, 18, 19). Dabei vergrößert sich das Antheridium allmählich. Nach der sechsten Teilung sieht man im Antheridium 64 freie Kerne (Fig. 20), welche dann aber durch dünne, protoplasmatische Scheidewände in 64 Spermatomutterzellen getrennt werden. Über die Einzelheiten der Spermentwicklung werde ich bei nächster Gelegenheit ausführlich berichten.

Die Chromosomenzahl der bisher untersuchten Fucaceenspezies sind in der folgenden Tabelle zusammengestellt.

Pflanzennahme	Haploide Chromosomenzahl		Diploide Chromosomenzahl	Autor
	♀	♂		
<i>Fucus vesiculosus</i> L.	32	32	64	YAMANOUCHI (1909)
<i>Sargassum enerve</i> C. AG.	32	--	--	TAHARA und SHIMOTOMAI (1926)
<i>Cystophyllum sisymbrioides</i> J. AG.	32	--	—	SHIMOTOMAI (1928)
<i>Sargassum Horneri</i> C. AG.	16	16	32	KUNIEDA (1928)
"	32		--	OKABE (1929)
<i>Coccophora Langsdorffii</i> (Turn.) Grev.	32		—	TAHARA (1929)
"			64	TOMITA (1932)
<i>Pelvetia Wrightii</i> (Harv.) YENDO	32	—		INOH (1933)
<i>Sargassum confusum</i> AG.	—	32	---	ABE (1933)

Wie obige Tabelle zeigt, besitzen manche Arten unter den Fucaceen nach neueren Untersuchungen verschiedener Forscher 32 reduzierte Chromosomen. KUNIEDA (1928), und nur er hat bei der Oogenese und der Spermatogenese bei *Sargassum Horneri* 16 haploide Chromosomen gezählt, aber sein Ergebnis wird durch OKABES Untersuchungen der Oogenese bei dieser Art und durch meine über die Mitosen im Antheridium von *Sargassum confusum* sehr zweifelhaft. Diese Nichtübereinstimmung ist wahrscheinlich durch die Fixierungsmethode veranlasst worden.

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### TAFELERKLÄRUNG.

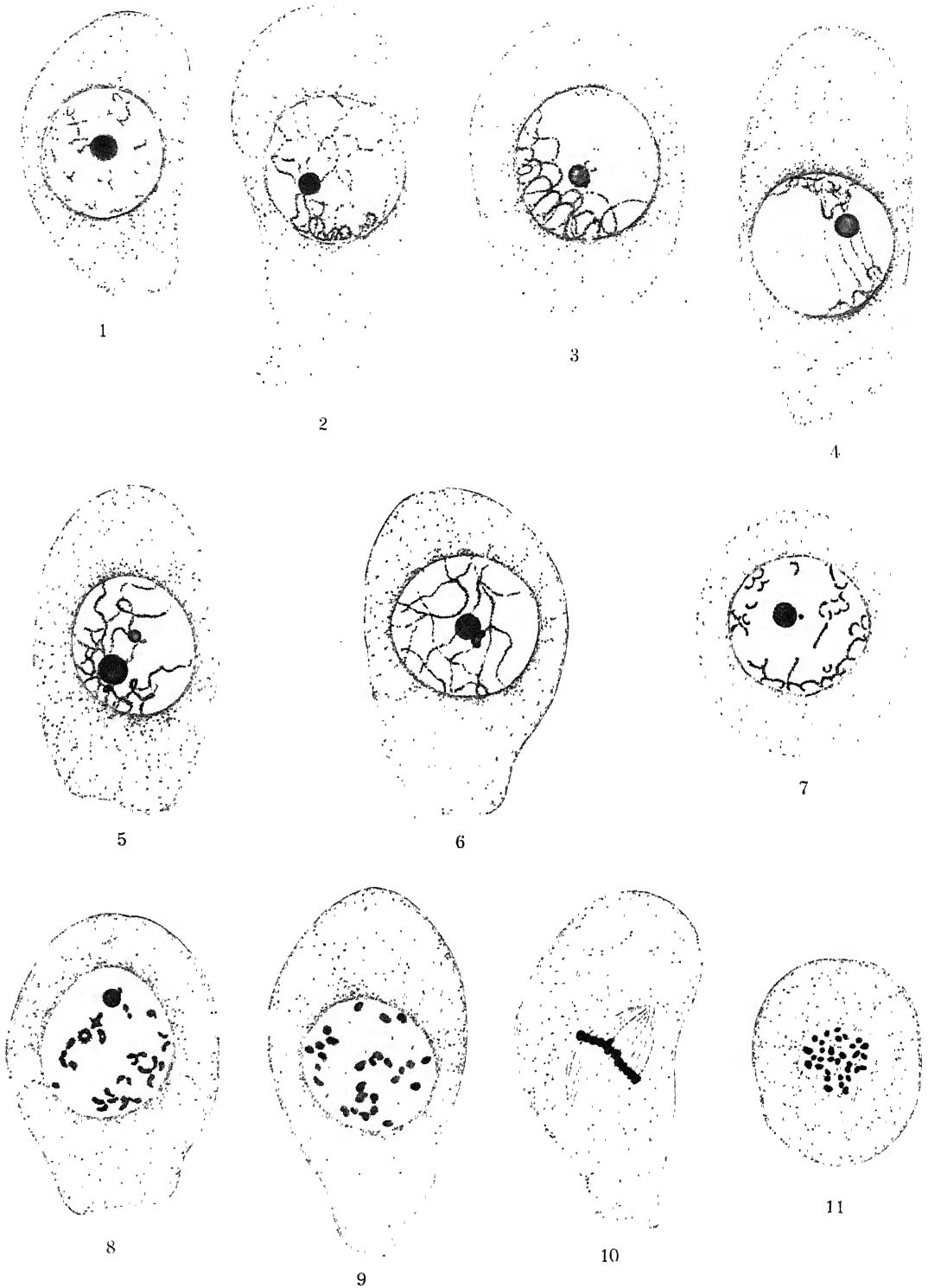
Alle Figuren wurden mit Hilfe eines ABBS'schen Zeichenapparat gezeichnet, unter Benutzung des LEITZ'schen Objektiv, Ölimmersion 1/12 und des ZEISS'schen Okular  $\times 17$ . Vergrößerung 2000.

### TAFEL IX.

- Fig. 1. Ruhestadium.
- Fig. 2-3. Früheres Synapsisstadium.
- Fig. 4. Dasselbe mit zwei synaptischen Knäueln.
- Fig. 5. Späteres Synapsisstadium.
- Fig. 6. Spiremstadium.
- Fig. 7-8. Frühere Diakinese.
- Fig. 9. Spätere Diakinese.
- Fig. 10. Heterotype Metaphase in Seitenansicht.
- Fig. 11. Dasselbe in Polansicht.

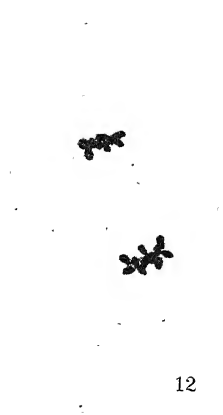
### TAFEL X.

- Fig. 12. Anaphase.
- Fig. 13. Telophase.
- Fig. 14. Homöotype Anaphase.
- Fig. 15. Homöotype Telophase.
- Fig. 16. Metaphase in der dritten Teilung.
- Fig. 17. 8 kerniges Stadium.
- Fig. 18. 16 kerniges Stadium.
- Fig. 19. 32 kerniges Stadium.
- Fig. 20. 64 kerniges Stadium.



K. ABE: Mitosen im Antheridium von *Sargassum Confusum*.





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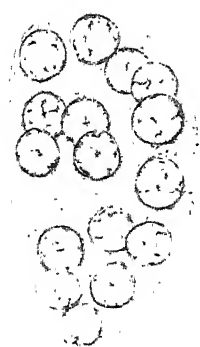
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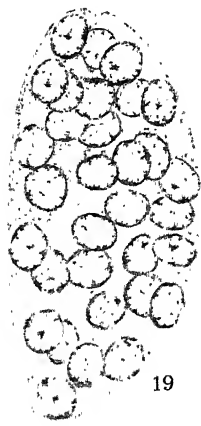
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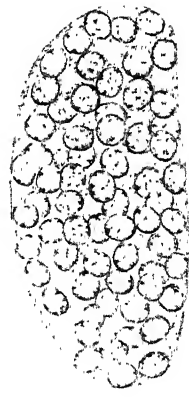
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# NOTES ON THE DEVELOPMENT OF THE SEA URCHIN *TEMNOPLEURUS HARDWICKII*.\*

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(With 20 text-figures)

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The following are some observations taken during the summer of 1933 on *Temnopleurus hardwickii*, a sea urchin common at Asamushi, Aomori-ken, Japan. Since it is the only sea urchin furnishing ripe eggs and sperm in quantity there during the summer months I have added some other general observations hoping they may be of value to workers using these eggs in the future.

## I.

The adult sea urchins after being dredged from the bottom of the bay should be kept in damp seaweed and as soon as possible placed in running sea water. If they are left in buckets of standing sea water the animals soon die. The water should flow freely over them in shallow dishes without carrying air bubbles. The sea urchins usually keep for two or three days after which new ones should be brought. If the room temperature reaches 26°C. they spawn spontaneously and so have to be discarded. The reproductive activity of the animals seems to follow a rhythm of about two weeks. For instance every animal may contain ripe sperm or eggs for a week, then fewer than one in ten the next week, but the week after more and more ripe testes or ovaries appear again until the maximum is reached. If one of these maxima coincide with a hot spell of weather, it will be some days before ripe eggs and sperm are found again because all the sea urchins in the bay will have spawned naturally.

The sea urchins can adjust themselves to abrupt changes in the salinity of the sea water. On one occasion a heavy rain altered the specific gravity of the laboratory running sea water from 1.0025 to 1.0015. This change

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\* Contribution from the Marine Biological Station, Asamushi, Aomori-ken, No. 104.

did not injure the adults, but the eggs taken from them and put into this diluted sea water failed to develop, or only produced irregularly dividing blastomeres. However after the sea urchins had remained for a day in the diluted sea water of the aquarium the development of the eggs in that water was normal, but they were then sensitive to sea water of higher specific gravity, since eggs of acclimatized sea urchins placed in sea water of original specific gravity (1.0025) do not develop when fertilized. However they will develop in sea water of lower sp. gr.

The pH of the sea water must be at least 8.0 for successful fertilization. In practice a few drops of n/10 alkali were added to a suspension of eggs to bring the pH to about 8.4 before sperm was added. After sperm was added the whole was gently agitated and the eggs a minute later transferred to about 50 cc. of filtered sea water in a finger bowl. Immediately upon impregnation a fertilization membrane forms. About twenty minutes later the inner hyaline membrane forms (Fig. 1, a).

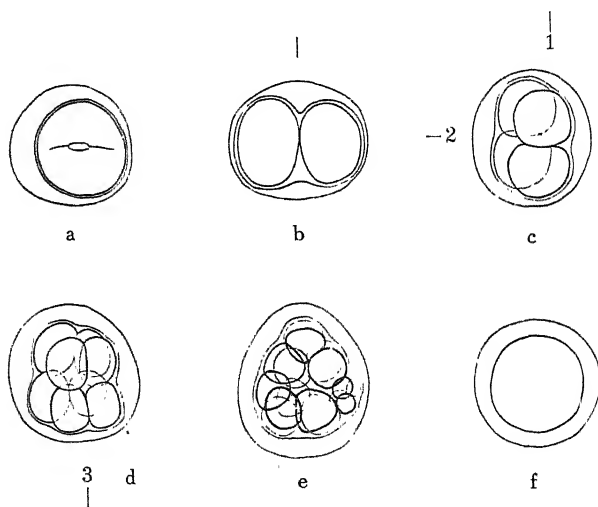


Fig. 1. Showing stages in the normal development of *Temnopleurus* eggs; a) to e), the same egg, the numbers indicating the first, second and third division planes. f), outline of a corresponding blastula after hatching.

Developing eggs are injured by temperatures of 26°C. and over, and by the low temperature of an ice-box.

The *Temnopleurus* egg is remarkably clear, the light brown pigment and yellow granules making it especially good for daylight observation. The whole drama of fertilization and cell division may be clearly seen.

The positions of the female and male pronuclei, their union, the enlargement of the resulting nucleus after formation, cytoplasmic streaming and final division of the nucleus and cytoplasm to form two cells, all are plainly visible. Cell boundaries are especially clearly seen by daylight, but the yellow light of an electric lamp obliterates them. Figure 1 shows camera lucida outlines of normally developing eggs.

The rate of division at ordinary temperature is shown in Table 1.

TABLE 1.

23°C.	From fertilization to first division,	53 minutes.
	From first to second division,	26 min.
	From second to third division,	27 min.
	From third to fourth division,	28 min.
25°C.	From fertilization to first division,	42 min.
	From first to second division,	22 min.
	From second to third division,	20 min.

About six hours after fertilization at ordinary temperatures the blastula begins to rotate within the fertilization membrane. The membrane becomes flattened and wrinkled in places and finally breaks, allowing the blastula to swim out. Once free the blastula rotates more rapidly and swims at the top of the dish of sea water (Fig. 1, f). About an hour after the blastulae begin to rotate, all of them have hatched and are spinning about the dish. At this time the mesenchyme cells are visible at the vegetative pole as they project slightly into the blastocoele. Eight hours after fertilization they are migrating into the cavity (Fig. 2). Two hours later, gastrulation has begun, and the mesenchyme cells have moved at least half way to the animal pole (Fig. 3). At 16 hours, the first skeleton has been formed, and gastrulation is complete (Fig. 4). At 24 hours, the tripartite gut has formed and the characteristic skeleton partially laid down (Figs. 5 and 6). At 2 days the mouth parts are complete (Fig. 7). At 3 days the pluteus has attained the external form it will have for about ten days (Figs. 8 and 9).

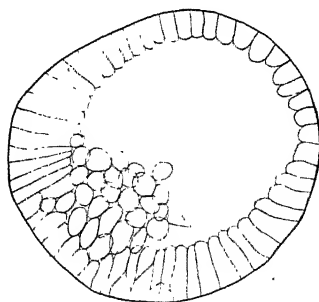


Fig. 2. Blastula eight hours after fertilization. Actual diameter, 0.1 mm.

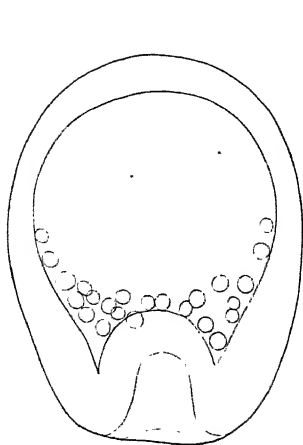


Fig. 3. Beginning gastrulation at ten hours.

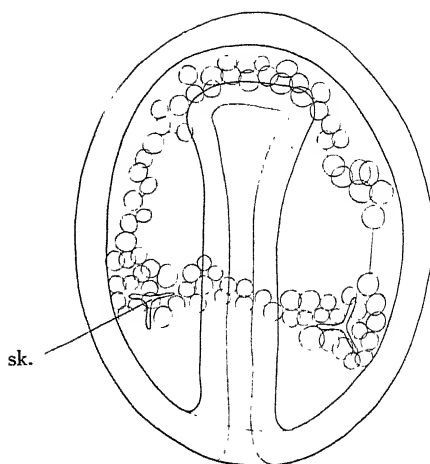


Fig. 4. Gastrula showing the first skeleton and the arrangement of the mesenchyme cells.

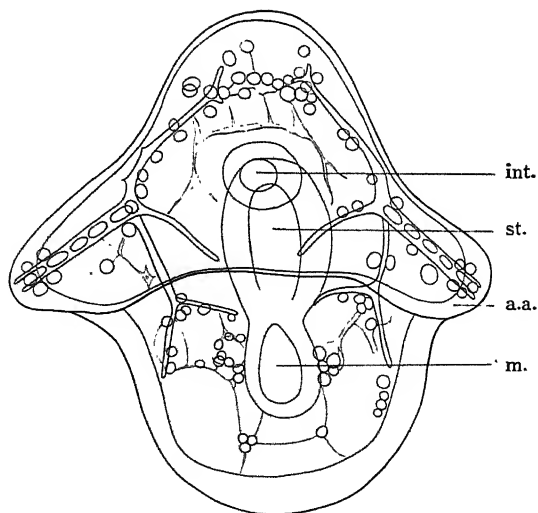


Fig. 5. Twenty-four hour larva with mouthparts still incomplete but with characteristic skeleton being secreted by the mesenchyme cells. Actual size 0.2 mm. across longest diameter.

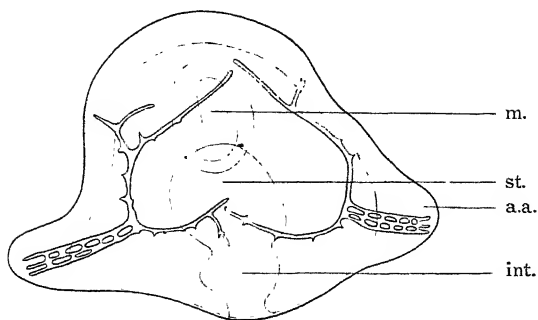


Fig. 6. Twenty-four hour larva viewed from above.

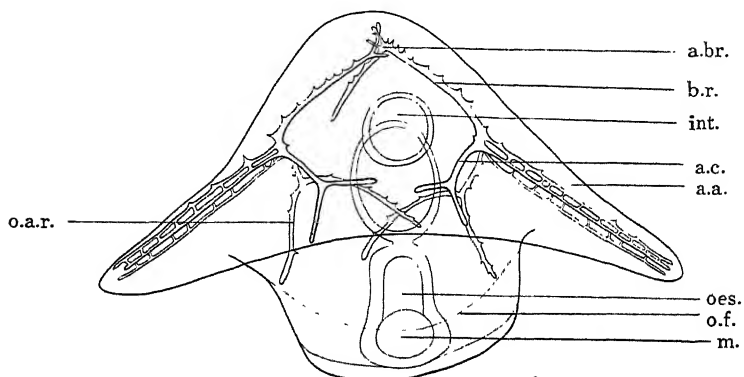


Fig. 7. Two day old pluteus viewed slightly from below.

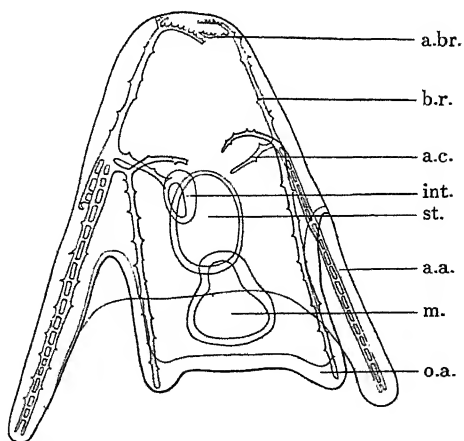


Fig. 8. Oral view of a three day old pluteus. Length excluding arms, 0.26 mm.

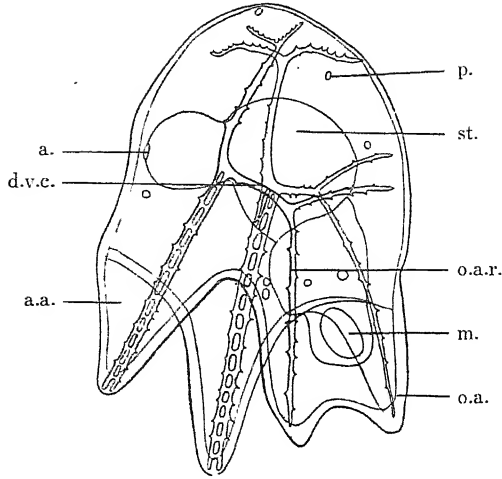


Fig. 9. Side view of a three day old pluteus. Actual length excluding arms, 0.26 mm.

## II.

*Temnopleurus* eggs resemble other sea urchin eggs in many of their chemical reactions. Experiments which have been done with both European and American forms can be repeated in *Temnopleurus* with only slight but instructive variations in the results. For instance the necessity of calcium ions for the formation of the hyaline membrane and the coherence of the blastomeres, first shown by HERBST<sup>1)</sup> on European forms, can easily be repeated here. If the newly fertilized eggs of *Temnopleurus* be taken up in a pipette of bore about the diameter of an egg, and then

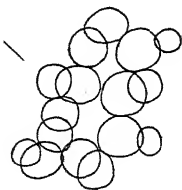


Fig. 10. Sixteen cell stage of an egg mechanically deprived of fertilization membrane and transferred to calcium free artificial sea water.

gently expelled, the fertilization membranes on many eggs will be broken away. If now these eggs are transferred through two or three changes of calcium-free sea water, and left to develop, no hyaline membrane will form. The two first blastomeres will also fall apart due to a breaking of the cell bridge, and later stages show groups of round cells barely touching each other. No blastula can form even if cell division itself is not halted from lack of calcium (Fig. 10).

Another way in which the eggs of *Tem-*

*temnopleurus* resemble those of other sea urchins is in their reaction to pure non-electrolyte solutions. The effect of pure urea or glycerine solution upon the fertilization and hyaline membranes was first described by MOORE<sup>23</sup> for the Pacific Grove echinoderms and later extended to Atlantic and European forms. Treatment with pure non-electrolyte solution may either prevent the formation of the fertilization membrane, or dissolve it after it has been formed in sea water. By putting the unfertilized eggs of *Temnopleurus* into a molecular solution of urea of pH 8.0 for three minutes the formation of the fertilization membrane is inhibited. For if these treated eggs are then transferred to sea water of pH 8.0 and sperm added, the eggs will be fertilized and will divide, but no fertilization membrane will form. About twenty minutes later the hyaline membrane will appear, however, and the eggs will develop into oval blastulae, and into plutei which will be hard to distinguish from normal ones. Normally formed fertilization membranes can also be dissolved off *Temnopleurus* eggs by transferring them immediately after fertilization and membrane formation, to a molecular urea solution of pH 8.0. As soon as the membranes have dissolved, the eggs must be transferred immediately back to sea water. However, as is the case with other sea urchins no hyaline layer appears after this treatment (Fig. 11).



Fig. 11. An egg almost at four cell stage dividing in sea water after treatment with urea solution to dissolve off the fertilization membrane. The cell bridge is too tenuous to hold cells together in the absence of the hyaline membrane.

*Temnopleurus* shows cell bridges<sup>23</sup> in appropriate stages after the fertilization membrane has been removed by chemical or mechanical means and whether the hyaline membrane is present or not. In *Temnopleurus* the cell bridges acting alone are too tenuous to hold the two blastomeres, or the first four, together to form an embryo in sea water (Fig. 11). Gentle agitation with an ordinary pipette will separate them. Consequently, depending upon how the blastomeres happen to lie with reference to each other, one or two or more blastulae of corresponding size will develop from them. If however, the hyaline membrane is present the blastomeres even if slightly separated will unite to form a slightly irregular but perfect larva (Fig. 12). In this form the cell bridges, even if the hyaline layer is present, may easily be observed in the two or four cell stage by using the slight pressure of a cover-slip placed upon a drop of sea water containing eggs. Those with fertilization membrane



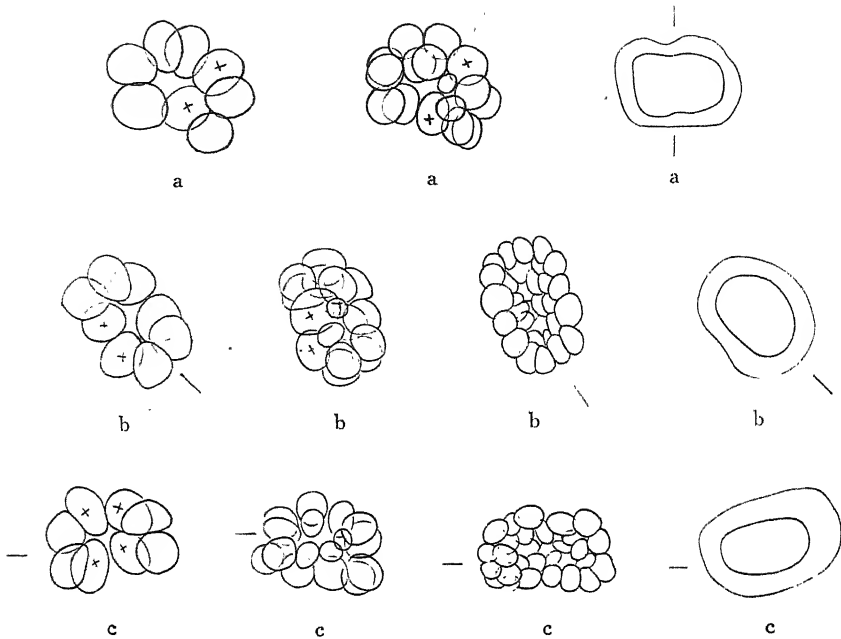


Fig. 12. Showing development in sea water of eggs deprived mechanically of fertilization membrane but with hyaline membrane intact. The dash shows the plane of the first cleavage furrow. The blastulae are slightly irregular. The  $\times$  indicates cells giving rise to micromeres.

removed mechanically but with hyaline membrane intact show beautiful primary and secondary cell bridges. The "spinning activities" of cells at the four and eight cell stages are very easily followed in such preparations (Fig. 13). If the hyaline layer is absent cell bridges are easily visible after the first, second, or third divisions.

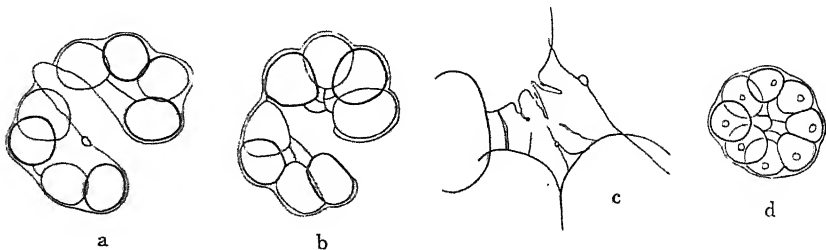


Fig. 13. Showing cell bridges. a) and b), successive drawings of the same egg. The fertilization membrane has been removed by pipetting and the hyaline membrane ruptured by slight pressure from the cover slip. c), The same, showing spinning activities of the blastomeres. d), Another eight cell stage showing cell bridges.

Since the first two blastomeres are easy to separate it is a simple matter to study the relation of the first cleavage plane to the plane of symmetry of the embryo. Any one of several methods of separation may be used (Fig. 14). An easy separation is made by removing the newly

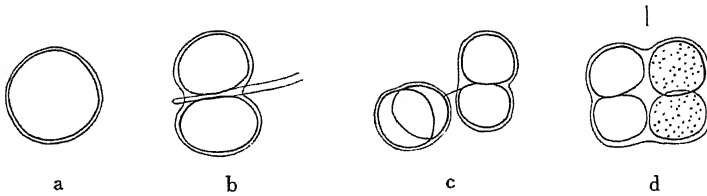


Fig. 14. Illustrating the methods of studying micromere formation in eggs having only the hyaline membrane, a). b), and c), mechanical separation of the first two blastomeres with a glass needle. d), the two blastomeres stained with Nile-sulphate-blue can clearly be distinguished from the other two.

formed fertilization membranes with a small bore pipette, as described above, and later when the first division is complete, by cutting through the hyaline membrane and cell bridge with an ordinary glass needle under the binocular microscope.\* If the eggs in the two cell stage are put into calcium-free sea water the separation is easier but the cells of later stages also separate from each other, making observations more difficult. If the fertilization membranes are dissolved by means of urea solution the first two blastomeres can also be easily separated by gentle agitation, and the segmentation of the two half eggs followed.<sup>4)</sup>

The segmentation of *Temnopleurus* eggs is equal up to the sixteen cell stage when as a rule four micromeres appear at the vegetal pole, four macromeres lie at the center and eight mesomeres at the animal pole. However, the micromere formation is very variable in *Temnopleurus*. Micromeres may be split off at the third division (eight cell stage) or may be deferred until the fifth. They are recognized by their clear protoplasm which includes few yellow granules, as well as by their small size. Not only the time of appearance of the micromeres, but the number of them is variable. Since they represent material which is responsible for the invagination of the blastula, and for the mesenchyme cells which

\* According to PLOUGH<sup>5)</sup> it is very difficult to separate the blastomeres of *Arbacia punctulata* even in calcium-free sea water by this method. The blastomeres of *Paracentrotus lividus* are easily separated in calcium-free sea water, but not in ordinary sea water, while those of *Strongylocentrotus purpuratus* and of *Temnopleurus hardwickii* are easily separated in ordinary sea water.

secrete the skeleton, it is interesting to find this latitude of variation in their formation in *Temnopleurus*. My studies based on the distribution of the micromeres between the daughter cells of the first two blastomeres show that the first cleavage may divide the future embryo symmetrically into right and left halves, or into dorsal and ventral halves or into animal and vegetal halves, or it may divide the future larva in any intermediate plane, i. e. obliquely with respect to the micromere-forming material. I used the well known method of staining one blastomere with a Nile-sulfate-blue agar plate while the eggs were otherwise undisturbed within their membranes (Fig. 14, d). In this way the daughter cells of the first two blastomeres could easily be distinguished. The results were the same as with the other methods. The actual number of daughter cells from both of the first two blastomeres was absolutely constant for each stage, i. e. there were always eight cells at the third division or sixteen at the fourth, whatever their size or appearance. No attempt was made to find the percentage of variation from the most usual, but in order to be sure that the experimental eggs were not injured they were followed to the stage of swimming blastulae. All the eggs from a given female tended to divide in the same way. If we call the two first blastomeres A and B, then Table 2 will show the variability of *Temnopleurus* with respect to its segmentation.

TABLE 2.

	Number of micromeres		Remarks
	A	B	
Eight cell stage	0	0	Even division 1 micromere, 7 larger cells
	1	0	
	2	0	
	2	2	
Sixteen cell stage	0	0	Micromeres in 32-cell stage
	1	0	
	1	1	
	2	0	
	2	2	
	3	1	
	3	2	
	3	3	
	4	0	
	4	1	Vegetal and animal halves

DRIESCH's early observations on the development of  $1/2$  blastomeres are easily repeated here. If the blastomeres of an egg are completely separated each will produce a blastula. But if one blastomere is killed

and both are held in a fertilization membrane, then the living one will produce an open blastula instead of a normal ball shaped one (Fig. 15).

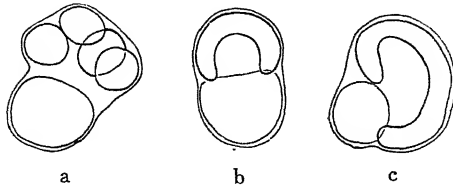


Fig. 15. Showing the formation of the open blastula when one of the first two a), b), or one of the first four blastomeres has been killed within the fertilization membrane, c).

### III.

It remains for other investigators to make a thoroughgoing study of the development of the echinoid element in the pluteus of *Temnopleurus*. This form is very suitable for such a study, since its culture is so easy. I put into each clean finger bowl about 20 vigorous plutei and 50 cc. of sea water freshly drawn in glass from the surface of the bay. I kept the bowls covered with glass plates, and transferred the plutei to clean bowls of fresh sea water every day. The plutei ate small algae and grew very well. About ten days after fertilization they developed a third pair of arms between the oral and the anal pair (Fig. 16 and Fig. 17). At

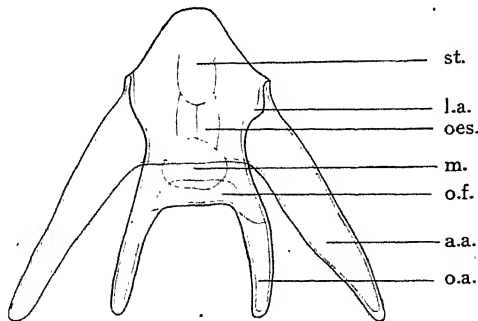


Fig. 16. Twelve day old pluteus showing the beginning of the lateral pair of arms. Actual length excluding arms is 0.34 mm.

this stage and thereafter their color was transparent green (Fig. 18). Later four ciliated epaulettes appeared at the base of the four large arms (the lateral and the anal pairs) (Fig. 19). These epaulettes are heavily ciliated and extend out at the sides, moving the larvae rapidly through

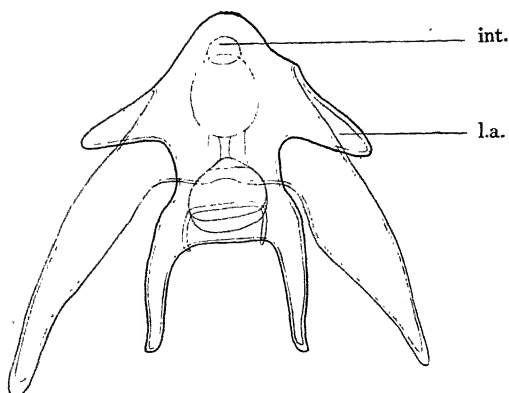


Fig. 17. Showing further growth of lateral arms.

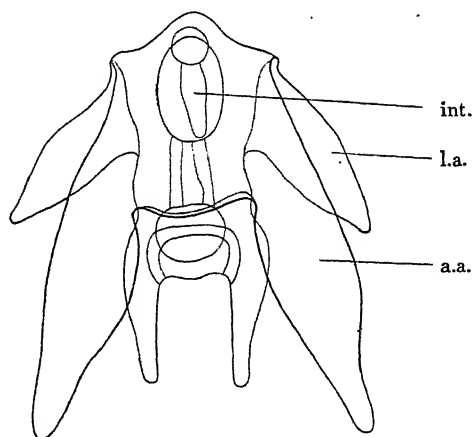


Fig. 18. Aboral view of a larger pluteus. From top to tip of longest arms, 0.78 mm.

the water. They now swim with all arms hanging down, so that viewed from the top the larvae appear rectangular. At the same time the fourth pair of arms (the second oral pair) develop. The echinoid element is visible from about the time the third pair of arms appears (Fig. 20). Unfortunately I left the station before metamorphosis actually occurred but in some of the larger plutei the echinoid element was so far developed that the characteristic adult markings and color were plainly seen through the transparent green larvae. All drawings were made from life with camera lucida. The preserved and mounted specimens while showing all

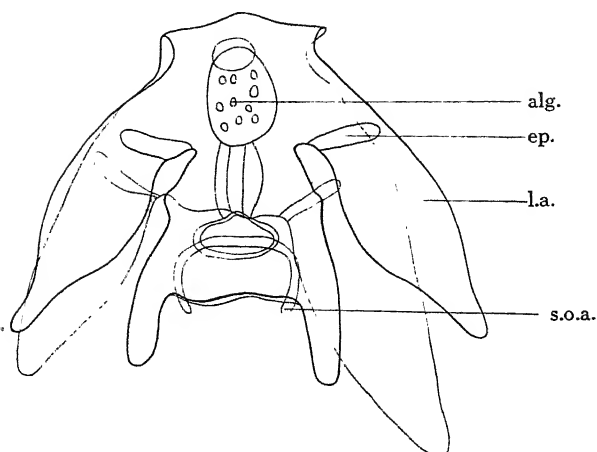


Fig. 19. Oral view showing lateral arms complete and the ciliated epaulettes partially developed. Also the beginning of the second pair of oral arms.

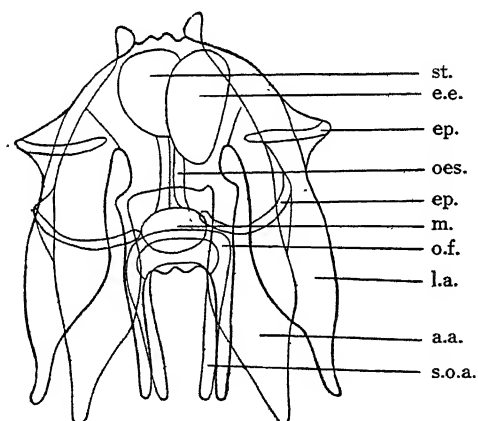


Fig. 20. A month old pluteus, showing the echinoid element greatly developed and the arms and the ciliated epaulettes fully developed. Oral view, total actual length, 3.0 mm.

the parts, never give a correct idea of what the living plutei are like, when cultured or taken in the plankton net.

In conclusion I wish to thank Dr. HATAI and the members of the Asamushi Station staff for their hospitality and kindness.

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## ABBREVIATIONS FOR ALL FIGURES.

<i>a.</i> ,	anus.
<i>a.a.</i> ,	anal arm.
<i>a.br.</i> ,	aboral branches.
<i>a.c.</i> ,	anal connective.
<i>alg.</i> ,	algae.
<i>b.r.</i> ,	body rod.
<i>dv.c.</i> ,	dorsoventral connective.
<i>e.e.</i> ,	echinoid element.
<i>ep.</i> ,	ciliated epaulettes.
<i>int.</i> ,	intestine.
<i>l.a.</i> ,	lateral arm.
<i>m.</i> ,	mouth.
<i>o.a.</i> ,	oral arm.
<i>o.a.r.</i> ,	oral arm rod.
<i>oes.</i> ,	oesophagus.
<i>o.f.</i> ,	oral flap.
<i>p.</i> ,	pigment.
<i>sk.</i> ,	skeleton.
<i>s.o.a.</i> ,	one of the second pair of oral arms.
<i>st.</i> ,	stomach.

# STUDIES ON ACID OF THE BODY FLUID FROM AN ASCIDIAN, *CHELYOSOMA SIBOJA* OKA.<sup>1)</sup>

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(With 1 figure)

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## INTRODUCTION

A high acid reaction in the body fluid or in the tissue is not only rare in the tunicates but also in animals in general.

M. HENZE (1911) has first reported the presence of acid reaction in the blood of an ascidian, *Phallusia mamillata*, found at Naples in the Mediterranean. He (1912) analysed the plasma and corpuscle separated from its blood and obtained the following ratios of  $\frac{SO_3}{Cl}$  : --

Blood corpuscle	Plasma	Sea water
2.55	0.0558 ( $\Delta=2.14$ )	0.1171 ( $\Delta=2.20$ )

HENZE concluded from the above, that the acid reaction of this blood due to the presence of free sulfuric acid in the corpuscle which amounts to ca. 3% (0.6 N.), is not present in the plasma.

Another ascidian, *Ascidia atra*, which also presents an acid reaction was found by S. HECHT (1917) at Bermuda in the Atlantic Ocean. He, however, demonstrated merely the presence of an acid reaction in the blood corpuscles but not in the plasma by testing with litmas paper. HECHT was prevented to perform the quantitative analysis of this blood, owing to obtain the insufficient quantity for this purpose.

In a later investigation, M. HENZE (1913) showed the presence of sulfuric acid in the mantle fluid of *Ascidia mentula*.

*Chelyosoma siboja* OKA is one of the large solitary fixed ascidians with the cartilaginous test commonly found in Mutsu Bay near the Marine Biological Station.

This species lives on a sandy or muddy bottom, 20-30 fathoms deep,

<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-ken, No. 105.

<sup>2)</sup> I am greatly indebted to Prof. S. HATAI in reading and criticizing the manuscript, and to Mr. S. SAWANO for the use of the apparatus.



attaching its base to stones or dead shells. The diameters of a medium sized specimen at the syphon plate measures 7.5 cm.  $\times$  5.0 cm. and 12.0 cm. in height. From a cut surface of the test, a brown coloured body fluid, containing the corpuscles drips out, which gives a strong acid reaction when tested with the pH indicator (thymol blue).

By gently pressing the body, even 40–50 cc. of the fluid may be collected from the cut-surface when the specimen was large. Under the microscope two kinds of corpuscles are readily visible; the green pigmented cells with the spherical granules, 10  $\mu$  in diameter, and the large non-pigmented cells, 50  $\mu$  in diameter.

This work was undertaken at the Marine Biological Station at Asamushi during the summer of 1933, to determine the nature and amount of acid contained in the body fluid in *Chelyosoma siboja* OKA and the results so far obtained are presented in this report.

#### MATERIAL AND METHOD

The materials were collected by a dredge net, four miles off the station and were kept in a pool.

The following determinations were performed of the body fluid:— the amount of the corpuscles in volume, the freezing point by the BECKMAN's thermometer, hydrogen ion concentration by the hydrogen electrode, total acidity by titrating with 0.1 N. NaOH by phenolphthalein, quantitative chemical analysis of some anions in relation to its acidity, and the electro-metric titration of the fluids.

The amount of the corpuscles was measured by centrifuging the fluid for 20 minutes, which rotates 3000 per minute.

For the quantitative analysis of anions related to the acid radicals, the following methods were employed: WHITEHORN method for  $\text{Cl}'$ , YOUNGBURG's method of inorganic phosphate for  $\text{PO}_4'''$ , and for the determination of inorganic  $\text{SO}_4''$ , the protein in the body fluid was removed by precipitating with tungstic acid by the method of FOLIN and WU. The sulphate in the protein free filtrate was precipitated as benzdine sulphate by the procedure of KAHN and LIEBOFF and then titrated volumetrically with 0.02 N. NaOH.

The determinations measured above were performed on the body fluid, the plasma, the fluids obtained from the corpuscles by plasmolysis after freezing or adding distilled water.

The body fluid collected from 10–13 individuals was employed for each

analysis and a cut was always made at the ridge on the posterior end of the ascidian.

The fluid was drawn into the glass vessel attaching the cut-surface on the wall of the vessel.

From 100–130 cc. fluid thus collected, the first 10 cc. was used to measure the volume of the corpuscles, and another 10 cc. was diluted with distilled water to 100 cc. in the volumetric flask for the analysis. The remainder was centrifugalized to separate the corpuscles suspended in the fluid. The plasma thus separated was used for further analysis. 10 cc. of the sedimented corpuscles were diluted with distilled water to 100 cc. in the volumetric flask and the remainder of the corpuscle was freedzed to obtain the corpuscle fluid. In these analysis, the centrifugalized corpuscles were not washed, owing to prevent the diffusing out of acid into the wash-medium.

The fluids thus prepared, were filtered before use. The sea water was collected for analysis where the ascidians were found.

The electrometric titration was performed both in the plasma and in the ten-fold dilution with distilled water of the corpuscle.

#### EXPERIMENTAL RESULTS

1. *Determination of the volume of the corpuscle, Freezing point depression, Hydrogen ion concentration, Total acidity, and Quantitative analysis of  $Cl'$ ,  $SO_4''$  and inorganic phosphorus.*

As will be seen from Table I and IV, the volume of the corpuscles contained in the ascidian fluid is found to be ca. 40%. The freezing point depression is 2.02 in the plasma and 2.05 in the corpuscle, while that of the sea water is 1.98. The ascidian fluids are thus found to be either isotonic or hypertonic against sea water.

It was found that the plasma gave pH 1.80 and the corpuscle, pH 0.39. The pH of the ten-fold diluted fluid of the corpuscle gave pH 1.27 and that of the body fluid, pH 1.33, indicating that the acidity of the latter is less than the former.

It is interesting to note in this connection that the pH of the sea water was found to be 8.2 by the indicator method (thymol blue).

The total acidity was found to be 0.027 N. in the plasm, 0.36 N. in the body fluid, 0.88 N. in the corpuscle-fluid obtained by the freezing method and 0.82 N. in the corpuscle-fluid by dilution with distilled water.

TABLE I.

Case of experiment	No. of individuals	Volume concentration of corpuscle. %		Freezing point depression. $\Delta$	Acidity		SO <sub>4</sub> g. in 1000 cc.	Cl g. in 1000 cc.	Inorganic phosphorus mg. in 1000 cc.
					pH	Normality			
I	11	42.4	Plasma	2.03	1.67	0.032	4.87	17.55	4.9
			Body fluid	—	1.54	0.35	20.35	10.28	4.8
			Corpuscle (diluted)	—	1.17	0.80	45.36	0.11	2.0
			Corpuscle (freezing)	—	0.41	0.88	47.47	2.12	4.8
II	12	33.3	Plasma	2.08	1.80	0.026	4.56	18.79	3.6
			Body fluid	—	1.56	0.35	23.04	10.65	3.9
			Corpuscle (diluted)	—	1.35	0.89	49.92	0.10	2.1
			Corpuscle (freezing)	—	0.26	0.98	55.29	1.77	3.4
III	13	41.7	Plasma	2.11	1.78	0.021	3.95	18.61	4.2
			Body fluid	—	1.50	0.36	23.50	10.63	4.1
			Corpuscle (diluted)	—	1.39	0.84	42.24	0.13	1.9
			Corpuscle (freezing)	—	0.51	0.90	54.72	2.30	3.8
IV	13	40.0	Plasma	1.98	1.93	0.022	4.90	17.50	4.3
			Body fluid	—	1.34	0.35	18.33	10.28	4.1
			Corpuscle (diluted)	—	1.20	0.78	45.12	0.13	1.9
			Corpuscle (freezing)	—	0.44	0.82	48.96	2.02	4.3
V	10	37.0	Plasma	1.98	1.78	0.034	6.53	17.50	4.3
			Body fluid	—	1.59	0.40	22.08	10.63	3.7
			Corpuscle (diluted)	—	1.24	0.79	45.60	0.10	1.5
			Corpuscle (freezing)	—	0.31	0.80	47.52	2.48	3.5
Sea water	—	—	—	1.98	8.2	—	2.66	19.14	Trace

All these values obtained from the ascidian fluids given above are higher than those found from the sea water. The values of the hydrogen ion concentration and of the total acidity arrange in the following order of magnitude:

Corpuscle > Body fluid > Plasma.

Among the anions which are expected to be related to acidity in the ascidian fluid,  $\text{SO}_4$  was lowest (4.96 g. in 1000 cc.) in the plasma, highest (50.79 or 45.65 g. in 1000 cc.) in the corpuscle-fluids and the medium value (21.46 g. in 1000 cc.) was given by the body fluid. The amount of  $\text{SO}_4$  given by the corpuscle-fluid formed by distilled water was slightly lower (45.65 g. in 1000 cc.) than those formed by the freezing method (50.79 g. in 1000 cc.). The  $\text{SO}_4$  of sea water (2.66 g. in 1000 cc.) was found to be decidedly lower than those formed in all the ascidian fluids given in the above. Cl was highest (17.99 g. in 1000 cc.) in the plasma, lowest (2.14 or 0.12 g. in 1000 cc.) in the corpuscle-fluids and the medium value (10.49 g. in 1000 cc.) was given by the body fluids. The amount of Cl given by the corpuscle formed by distilled water (0.12 g. in 1000 cc.) was found to be remarkably lower than those formed by the freezing method. The Cl of the sea water (19.15 g. in 1000 cc.) was found to be usually higher than those in all the ascidian fluids.

The  $\text{SO}_4$  and Cl contents of the ascidian fluids, compared with those of the sea water are shown in Table II.

TABLE II.

	$\frac{\text{SO}_4 \text{ in ascidian fluid}}{\text{SO}_4 \text{ in sea water}}$	$\frac{\text{Cl in ascidian fluid}}{\text{Cl in sea water}}$
Plasma	1.86(1.71-2.46)	0.94 (0.91-0.98)
Body fluid	8.82(7.65-10.65)	0.55 (0.54-0.56)
Corpuscle fluid (10 fold diluted)	17.17(15.88-18.77)	0.11 (0.09-0.13)
Corpuscle fluid (freezing)	19.10(17.75-20.46)	0.006(0.005-0.007)

The data in the bracket denote the extremity of the determinations.

The ratios between  $\frac{\text{SO}_4}{\text{Cl}}$  obtained from the ascidian fluids and from the sea water at Asamushi are shown in Table III.

TABLE III.

	Ratios of $\frac{\text{SO}_4}{\text{Cl}}$ in the ascidian fluids and sea water.			
	Corpuscle fluid	Body fluid	Plasma	Sea water
HENZE	2.55	—	0.0558	0.1171
Writer	20.14 (freezing) 333.7 (plasmolysis)	1.86	0.231	0.1158

The phosphorus content in mg. in 1000 cc. was determined to be 4.2 in the plasma, 4.1 in the body fluid, and 3.9 in the corpuscle-fluid by freezing. The corpuscle fluid caused by plasmolysis with distilled water gave a lower value (1.9 mg. in 1000 cc.) than that of plasmolysed by the freezing method as were the cases found in the determination of  $\text{SO}_4$  and  $\text{Cl}$ .

Accurate determination of the sea water is difficult owing to the appearance of the yellow colour. The results obtained are summarized in Table IV.

TABLE IV.

	$\Delta$	pH	Total acidity	$\text{SO}_4$ g. in 1000 cc.	$\text{Cl}$ g. in 1000 cc.	P mg. in 1000 cc.
Plasma	-2.02 (1.98-2.08)	1.80 (1.67-1.98)	0.027 N (0.021-0.034)	4.96 (4.87-6.53)	17.99 (17.50-18.79)	4.2 (4.9-3.6)
Body fluid		1.54 (1.44-1.59)	0.36 N (0.35-0.40)	21.46 (18.33-23.50)	10.49 (10.28-10.65)	4.1 (4.80-3.7)
Corpuscle (diluted)		1.27 (1.17-1.39)	0.82 N (0.78-0.89)	45.65 (42.24-49.92)	0.12 (0.10-0.13)	1.9 (2.0-1.5)
Corpuscle (freezing)	-2.05 (1.98-2.14)	0.38 (0.26-0.51)	0.88 N (0.82-0.98)	50.79 (47.47-54.72)	2.14 (1.77-2.48)	3.9 (4.8-3.4)

The data in the bracket denote the extremity of the determinations.

## 2. *Electrometric titration of the ascidian fluids using hydrogen electrode.*

In this experiment, the writer attempted to determine qualitatively the degree of change in acidity of fluids during the course of the titration by a strong base 0.1 N. NaOH, and also the nature of acid responsible for this reaction.

The ten-fold diluted fluid of the corpuscle with distilled water and non diluted centrifugalized plasma fluid were prepared for the electrometric titration.

These fluids so prepared were filtered and 10 cc. of each sample titrated with the standard strong base solution 0.1 N. NaOH, noting the change of pH immediately after a definite amount of titration solution was added. 0.1 N. sulfuric acid was also titrated with 0.1 N. NaOH for comparison.

The data of pH change of the ascidian fluids against the cc. of the standard base solution and of 0.1 N. sulfuric acid are shown in Table V and plotted in Fig. 1.

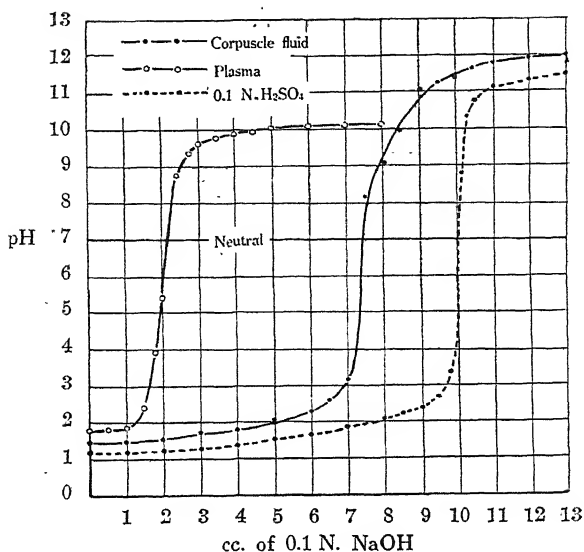


Fig. 1. Titration curves of ascidian fluids as compared with that of 0.1 N. sulphuric acid, obtained by hydrogen electrode.

As will be seen from Table V and Fig. 1, before the titration the hydrogen ion concentration in these three acid fluids and solution are pH 1.45 for the corpuscle fluid, pH 1.74 for the plasma and pH 1.15 for 0.1 N. sulfuric acid.

On titrating the ascidian corpuscle-fluid the change of the hydrogen ion concentration is very slight and only slowly reaches the point of pH 3.0 even when a large amount of the standard base solution is added. When, however, the reaction changes to pH 3.0, subsequent change becomes very rapid even when a very small amount of the solution added and reaches pH 7.0 or neutralization point. The same phenomenon just mentioned takes place with the titration of sulfuric acid.

In the ascidian fluids we find that from pH 7.0 to pH 11.0, a sharp increase of pH is prevented by some substances formed following the titration, which repress the ionization of the base and thus differs from the titration of 0.1 N. sulfuric acid. With the plasma fluid, the beginning value of pH is higher and the subsequent changes of pH are very rapid with a small addition of the base as with the former two cases, but at the point of pH 10, the titration with the large amount of the strong base solution did not produce any remarkable change of pH, probably due to the buffer action of this fluid.

TABLE V.

cc. of 0.1 N. NaOH	pH value		
	Plasma	Corpuscle	0.1 N. H <sub>2</sub> SO <sub>4</sub>
0.00	1.74	1.45	1.15
0.50	1.88	—	—
1.00	1.91	1.50	1.20
1.50	2.33	—	—
1.75	3.09	—	—
2.00	5.04	1.55	1.25
2.40	8.76	—	—
2.70	9.32	—	—
3.00	9.64	1.72	1.39
3.50	9.78	—	—
4.00	9.86	1.79	1.42
4.50	9.93	—	—
5.00	10.03	2.09	1.57
6.00	10.08	2.26	1.66
7.00	10.12	3.14	—
7.50	—	8.81	—
8.00	10.17	9.62	2.04
8.50	—	9.91	2.28
9.00	—	10.98	2.38
9.50	—	11.22	2.70
9.80	—	—	3.31
10.00	—	11.38	—
10.10	—	—	7.80
10.25	—	—	10.34
10.50	—	11.64	10.77
11.00	—	11.82	11.15
12.00	—	11.90	11.80
13.00	—	11.96	11.50

From these results, the curves of the ascidian fluid obtained by this method may be considered as essentially the same with that obtained from the titration of the strong acid with the strong base. It is therefore concluded that the high acidity found in the ascidian plasma and corpuscle is due to a presence of the strong acid and not a weak acid nor the mixture of these acids.

#### SUMMARY

The above experiments show that the osmotic pressure of the ascidian fluids is almost equal to or slightly higher than that of the sea water, and the acidity is high in the corpuscle, low in the plasma, and the body fluid stands between them.

The SO<sub>4</sub> contents of the ascidian fluid show the same order as with the relation found in acidity but the Cl contents show the reverse order; that is, low in the corpuscle-fluid and high in the plasma.

Phosphorus content is found to be equal in the three different kinds of the ascidian fluids and the amount of phosphoric acid estimated from the inorganic phosphorus actually found would be too small to be accounted for strong acidity of the ascidian fluids.

The above findings are further supported by the results obtained from the electrometric titration of the ascidian fluids.

The concentration of sulfuric acid in the ascidian fluids is equivalent to 0.88 N. or 4.3% in the corpuscle-fluid, 0.36 N. or 1.8% in the body fluid and 0.027 N. or 0.13% in the plasma.

The  $\text{SO}_4$  contents of the ascidian fluids are always greater and its Cl contents always less than that of the sea water, especially in the corpuscle.

The phosphorus contents of these fluids are found to be very high compared with that of the sea water.

Although the ratios between  $\frac{\text{SO}_3}{\text{Cl}}$  in the sea water at Asamushi agrees with that of the Mediterranean sea water at Naples, but those obtained from the ascidian fluid show a remarkable difference in the ones living in Mutsu Bay (*Chelyosoma siboga* OKA) compared to the other living in the Mediterranean (*Phallusia mamillata*).

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# THE DIAGNOSES OF THE POLLEN OF FOREST TREES. I.<sup>1)</sup>

By

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(With Pls. XI and XII)

(Received October 23, 1933)

Descriptions, atlases and keys of the pollen of European and American plants for use in pollen-analysis have been published by DOKTUROWSKY and KUDRJASCHOW<sup>2)</sup>, ERDTMAN<sup>3)</sup>, MEINKE<sup>4)</sup>, SEARS<sup>5)</sup>, etc. However, more or less incompleteness is found in their descriptions and illustrations, and it is of great importance to have a precise investigation of the pollen of our living plants before entering on pollen-analytical studies of peat or related materials of our country, to which little attention has hitherto been directed<sup>6)</sup>.

Since I have been working along this line, I intend to describe and illustrate here for purposes of pollen-analysis the diagnostic characters of the pollen of the main forest trees of Japan. Some cultivated trees of exotic origin were also investigated for reference.

Most of the material on which this study is based were obtained by myself from the city of Sendai and its environs, Mt. Hakkôda and Saghalin, and I am indebted to Dr. S. AKABAYASHI, head of the Sendai Forestry Office, by whose kindness I was enabled to obtain much valuable material from the garden of the Office and state forests at various spots in our country. Acknowledgments are also due to Dr. A. KIMURA, Dr. S. FUJISHIMA, Dr. S. CHÛHACHI, Dr. E. SAWANO, Dr. Y. TORYU, Dr. Y. HORIKAWA, Mr. M. KÔNO, Mr. K. NAGASHIMA, Mr. Y. MOMIYAMA and many

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<sup>1)</sup> Contributions from the Mt. Hakkôda Botanical Laboratory. No. 19.

<sup>2)</sup> DOKTUROWSKY, W., and KUDRJASCHOW, W., (1923). Schlüssel zur Bestimmung der Baumpollen im Torf. *Geol. Arch.*, 3, 180.

<sup>3)</sup> ERDTMAN, G., (1924). Beitrag zur Kenntnis der Mikrofossilien in Torf und Sedimenten. *Arkiv för Botanik*, 18, 14:1.

<sup>4)</sup> MEINKE, H., (1927). Atlas und Bestimmungsschlüssel zur Pollenanalytik. *Botan. Arch.*, 19, 380.

<sup>5)</sup> SEARS, P. B., (1930). Common fossil pollen of the Erie Basin. *Botan. Gaz.*, 89, 95.

<sup>6)</sup> Recently, T. YAMAZAKI published an atlas of the pollen of a number of plants, both woody and herbaceous, found in woods and moors in Saghalin. [*Rep. Exper. Forest Kyôto Imp. Univ.*, 5 (1933)]

other gentlemen, for their kind assistance given in collecting material.

I wish to express my cordial thanks to Prof. Dr. Y. YOSHII, under whose direction this work was made.

### *Methods.*

Dry pollen grains set free from the anther are generally shrunk or collapsed. Round pollen often assumes a cup-shape, half of the surface being sunken. They swell up when put into water or glycerine, the strongly swelling intine<sup>1)</sup> playing a great rôle in it, so that those which have a thin exine and no pores often burst.

On the other hand, the fossil pollen found in peat is empty as a rule, consisting only of the exine preserved after the protoplasm and the intine have decayed. The empty pollen found in preparations made from peat in the usual way<sup>2)</sup> is not so swollen as the fresh pollen simply mounted in water, glycerine or related media.

For the purpose of the present work it is essential to describe the pollen in a state similar to the preparations made from peat.

In this connection, I found that fresh pollen in general can easily be emptied by treating with concentrated hydrochloric acid for a very short time: Fresh pollen grains are put into a drop of the acid on a slide, then the acid is evaporated by gentle heating and neutralized by addition of a drop of about 10 per cent solution of potassium hydroxide. After heating the slide to make the drop evaporate to dryness, the pollen grains are moistened with glycerine and then investigated. The pollen grains thus treated are almost empty in general, without showing any change even in the minute structure of the exine. The intine is destroyed, as a rule, by this treatment. It seems to me that fresh pollen treated by this method can practically represent the fossil one in the preparation.

### *Description.*

The present description refers to the pollen taken from living plants exclusively, dealing only with the exine.

The size of the pollen of a single species is more or less variable and

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<sup>1)</sup> To detect the intine, an aqueous solution of ruthenium red is very useful, staining it very well. Methylene blue can also be used instead. The minute structure of the exine can sufficiently be observed without any stain.

<sup>2)</sup> We usually digest the material of peat with a solution of potassium hydroxide and mount it in glycerine.

here the range of variation found in the scope of my investigation is shown. In pollen of compressed or elongated shape, the diameter at the equator is given.

So called *expansion folds* mean lens-shaped thin-walled areas in the exine, such as are found for example in *Quercus*. They are usually folded up in shrunk pollen, so that the pollen looks rather tripetalous-flower-shaped in polar view in the case of *Quercus*, etc.

Species given below the generic name are those investigated.

As a rule, distinction between species within a genus is impossible; sometimes even different genera cannot be distinguished as in Cupressaceae.

## Taxaceae

### TAXUS

*Taxus cuspidata* SIEB. et ZUCC. (いちむ); — var. *ambraculifera* MAKINO (きゅうぼく).

SHAPE: roundish tetrahedron (without pores). SURFACE: very finely reticular (rather indistinct). SIZE: 20—28  $\mu$  in diameter.

### TORREYA

*Torreya nucifera* SIEB. et ZUCC. (かや).

SHAPE: roundish\* (without pores). SURFACE: very finely reticular (rather indistinct). SIZE: 24—30  $\mu$  in diameter.

## Cephalotaxaceae

### CEPHALOTAXUS

*Cephalotaxus drupacea* SIEB. et ZUCC. (いねがや).

SHAPE: roundish\* (without pores). SURFACE: with very small granular protuberances. SIZE: 25—32  $\mu$  in diameter.

## Pinaceae

### PINUS

*Pinus densiflora* SIEB. et ZUCC. (あかまつ); — *pentaphylla* MAYR. (こえふまつ); — *pumila* REGEL (ぼひまつ); — *Thunbergii* PARL. (くろまつ).

SHAPE: ellipsoid, with two roundish AIR SACS attached to both ends

\*. The distinct polyhedral character such as in *Taxus* cannot be ascertained here.

of the "ventral" side, (without pores). The EXINE is thickened on the "dorsal" side. SURFACE: finely reticular on the "dorsal" side, smooth on the "ventral" side; the surface of the air sacs is roughly reticular. SIZE: 60—85  $\mu$  in length (including the air sacs), the longer axis of the pollen proper exclusive of the air sacs is 40—62  $\mu$  long and the shorter axis 30—50  $\mu$ .

#### PICEA

*Picea jezoensis* CARR. (えぞまつ); — var. *hondoensis* REHD. (たうひ);  
— *polita* CARR. (はりもみ).

Identical with *Pinus* except for larger size — 100—120  $\mu$  in length (including the air sacs), the longer axis of the pollen proper is 75—90  $\mu$  long and the shorter axis 60—75  $\mu$ .

#### ABIES

*Abies firma* SIEB. et ZUCC. (もみ); — *Mariesii* MAST. (あなもりとどまつ);  
— *Mayriana* MIYABE et KUDO (あなとどまつ).

Identical with *Picea* and *Pinus*, except for still larger size — 125—140  $\mu$  in length (including the air sacs), the longer axis of the pollen proper is 85—105  $\mu$  long and the shorter axis 75—90  $\mu$ . The sculpture on the surface is more rough in *Abies* than in *Picea*, corresponding to the difference in the size of the whole pollen; it is the case also between *Picea* and *Pinus*.

#### TSUGA

*Tsuga diversifolia* MAST. (こめつか\*); — *Sieboldii* CARR. (つか\*).

SHAPE: compressed sphere, with a girdle of AIR SAC around the equator; differentiation into "dorsal" and "ventral" sides is found, the "ventral" side is smaller in area than the "dorsal" and sinks in dry state so that the whole pollen then appears cup-shaped, (pores are lacking). SURFACE: the whole surface is covered by relatively small sharp spines; the "dorsal" side shows winding wrinkles forming crack-like grooves, it is the case also on the "ventral" side but not so conspicuous; the air sac shows only sparse narrow wrinkles. SIZE: 55—100  $\mu$  in diameter at the equator (including the air sac).

#### LARIX

*Larix Kaempferi* SARG. (からまつ).

SHAPE: roundish (without pores); cup-shaped in dry state. The EXINE is thickened in a narrow band equatorially at the edge of cup-shaped dry pollen, frequently convex one of the two parts of surface bordered by the thickening is further halved by another similar thickening. SURFACE: almost smooth. SIZE: 75—95  $\mu$  in diameter.

## PSEUDOTSUGA

*Pseudotsuga japonica* BEISSN. (とがさばら).

Identical with *Larix* in the main, but here the SURFACE is finely reticular (rather indistinct) and the SIZE is 90—110  $\mu$  in diameter.

## Taxodiaceae

## CRYPTOMERIA

*Cryptomeria japonica* D. DON (すき).

SHAPE: spherical; cup-shaped in dry state with a pore situated at the centre of the concave surface. PORE: 1, surrounded by a ligulate projection from the exine. SURFACE: with very small granular protuberances. SIZE: 30—35  $\mu$  in diameter.

## SCIADOPITYS

*Sciadopitys verticillata* SIEB. et ZUCC. (かうやまき).

SHAPE: spherical (without pores). SURFACE: covered by irregular nodular protuberances except for a smooth round area less than a half of the whole surface — the smooth area sinks in dry state the whole pollen being cup-shaped. SIZE: 35—40  $\mu$  in diameter.

## CUNNINGHAMIA

*Cunninghamia sinensis* R. BR. (くわえふざん).

SHAPE: spherical (without pores). SURFACE: with very small granular protuberances. SIZE: 30—38  $\mu$  in diameter.

## Cupressaceae

## CHAMAECYPARIS

*Chamaecyparis obtusa* SIEB. et ZUCC. (ひのき); — *pisifera* SIEB. et ZUCC. (さばら).

## JUNIPERUS

*Juniperus chinensis* L. (びやくしん); — *conferta* PARL. (はひれす); — *rigida* SIEB. et ZUCC. (ねす).

## THUJA

*Thuja Standishii* CARR. (くろべ).<sup>1)</sup>

## THUJOPSIS

*Thujopsis dolabrata* SIEB. et ZUCC. (あすなろ).

The pollen of those genera of Cupressaceae is identical with that of *Cunninghamia*, viz. — SHAPE: spherical (without pores); SURFACE: with very small granular protuberances; SIZE: 27—33  $\mu$  in diameter in *Chamaecyparis*, 21—26  $\mu$  in *Juniperus*, 25—30  $\mu$  in *Thuja*, and 30—35  $\mu$  in *Thujopsis*.<sup>2)</sup>

Betulaceae<sup>3)</sup>

## ALNUS

*Alnus alnobetula* HARTIG var. *fruticosa* WINKL. (みやまはんのき); — *firma* SIEB. et ZUCC. var. *multinervia* REGEL (ひめやしゅぶし); — *japonica* SIEB. et ZUCC. (はんのき); — *tinctoria* SARG. (けやまはんのき); — — var. *glabra* CALL. (やまはんのき).

SHAPE: tetragonal or pentagonal, sometimes hexagonal, in polar view, elliptic in equatorial view. PORE: 4, 5 or 6 corresponding to the shape, situated at the angles, projecting. The EXINE appears dichotomous at the edges of the pores in the section. SURFACE: finely reticular (rather indistinct). SIZE: 20—30  $\mu$  in diameter,

<sup>1)</sup> Exotic species investigated, *Thuja occidentalis* L. (にほひびし) and *T. orientalis* L. (このてがし), form pollen quite similar to this species.

<sup>2)</sup> It is obvious that it may be impossible in pollen-analysis to identify the pollen of those genera of Cupressaceae, *Cunninghamia*, *Torreya* and *Cephalotaxus*, which has no remarkable character and, moreover, can easily be broken due to the thin wall.

<sup>3)</sup> Photomicrographs of the fossil pollen of some European trees taken by E. C. WASSINK [*Recueil des travaux botaniques néerlandais*, 29 (1932), p. 15 ff.] are to be referred to, with regard especially to that of *Betula*, *Corylus*, *Ulmus*, *Tilia* and *Fagus*, of which characteristics are clearly represented in them.

## BETULA

*Betula Ermanii* CHAM. var. *communis* KOIDZ. (だけかんば); — *latifolia* KOM. (しらかんば).

SHAPE: trigonal (very rarely tetragonal) in polar view, elliptic in equatorial view. PORE: 3 (or 4), situated at the angles, projecting. The EXINE appears dichotomous at the edges of the pores in the section. SURFACE: finely reticular (rather indistinct). SIZE: 30—35  $\mu$  in diameter in *Betula Ermanii* var. *communis* and 20—25  $\mu$  in *B. latifolia*.

## CORYLUS

*Corylus heterophylla* FISCH. var. *japonica* KOIDZ. (はしばみ); — *rostrata* AIT. var. *Sieboldiana* MAXIM. (つのはしばみ).

SHAPE: trigonal in polar view, elliptic in equatorial view. PORE: 3, situated at the angles, slightly projecting. The EXINE looks gradually thickened towards the edges of the pores, showing no distinct dichotomous edges — this character of the exine is of diagnostic value in distinguishing the pollen of *Corylus* from that of *Betula*. SURFACE: finely reticular (rather indistinct). SIZE: 25—30  $\mu$  in diameter.

## CARPINUS

*Carpinus carpinoides* MAKINO (くまじで); — *laxiflora* BLUME (あかしで); — *yedoensis* MAXIM. (いぬじで).

SHAPE: roundish in polar view (with projections at the pores), elliptic in equatorial view. PORE: 3 or 4, sometimes 5, situated on the equator in equal distances, projecting. The EXINE is relatively thin in a uniform thickness throughout. SURFACE: finely reticular (rather indistinct). SIZE: 25—35  $\mu$  in diameter.

## OSTRYA

*Ostrya japonica* SARG. (あさだ).

Identical with *Carpinus* (23—28  $\mu$  in diameter).

## Ulmaceae

## CELTIS

*Celtis sinensis* PERS. (えのき).

SHAPE: circular in polar view, elliptic in equatorial view. PORE: 3 (rarely



4), situated on the equator in equal distances. The EXINE is a little thickened at the edges of the pores. SURFACE: finely reticular (rather indistinct). SIZE:  $24-30\mu$  in diameter.

#### ULMUS

*Ulmus japonica* SARG. (はるにれ); — *parvifolia* JACQ. (あきにれ).

SHAPE: roundish — rather tetragonal or pentagonal (sometimes hexagonal) — in polar view, elliptic in equatorial view. PORE: 4 or 5 (sometimes 6), situated at the angles. The EXINE is not much thickened at the edges of the pores. SURFACE: very roughly wrinkled or very roughly reticular. SIZE:  $23-33\mu$  in diameter.

#### ZELKOWA

*Zelkova serrata* MAKINO (げやき).

SHAPE: tetragonal or pentagonal in polar view, elliptic in equatorial view. PORE: 4 or 5 corresponding to the shape, situated at the angles. The EXINE is considerably thickened at the edges of the pores. SURFACE: very roughly wrinkled. SIZE:  $35-40\mu$  in diameter.

#### Tiliaceae

##### TILIA

*Tilia japonica* SIMK. (しなのき).<sup>1)</sup>

SHAPE: roundish in polar view — often rather trigonal, a pore being situated at the middle of each side; elliptic in equatorial view. PORE: 3, oblong, situated on the equator in equal distances. Under the exine is found the particularly persistent intine which is much thickened inside the pores, a hollow cavity in connection with the pore appears enclosed in the thickening. SURFACE: very finely reticular. SIZE:  $30-37\mu$  in diameter.

#### Fagaceae

##### FAGUS

*Fagus crenata* BLUME (ぶな); — *japonica* MAXIM. (いぬぶな).

SHAPE: roundish in polar view, elliptic in equatorial view. EXPANSION

<sup>1)</sup> An exotic species investigated, *Tilia Miqueliana* MAXIM. (ぼだいじゅ), is the same as this species in pollen characters.

FOLD: 3, long. PORE: 3, situated at the middle of expansion folds. The EXINE is thickened at the edges of the pores. SURFACE: punctate. SIZE: 32—45  $\mu$  in diameter.

## QUERCUS

*Quercus acuta* THUNB. (あかかし); — *acutissima* CARR. (くめぎ); — *crispula* BLUME (みつなら); — *dentata* THUNB. (かしは); — *myrsinaefolia* BLUME (しらかし); — *serrata* THUNB. (こなら).

SHAPE: roundish in polar view, elliptic in equatorial view. EXPANSION FOLD: 3, long. PORE: 3, situated at the middle of expansion folds. SURFACE: punctate. SIZE: 23—35  $\mu$  in diameter.

## CASTANEA

*Castanea crenata* SIEB. et ZUCC. (くり).

SHAPE: roundish in polar view, elliptic in equatorial view. EXPANSION FOLD: 3, long. PORE: 3, situated at the middle of expansion folds. SURFACE: smooth. SIZE: 10—16  $\mu$  in diameter.

## Salicaceae

## POPULUS

*Populus Sieboldii* MIQ. (やまならし).<sup>1)</sup>

SHAPE: spherical (without pores). SURFACE: punctate. SIZE: 23—30  $\mu$  in diameter.

## SALIX

*Salix Bakko* KIMURA (ばっこやなぎ); — *integra* THUNB. (いねこりやなぎ); — *Reinii* FRANCH. et SAV. (みれやなぎ); — *sachalinensis* FR. SCHM. (なごやなぎ); — *vulpina* ANDERS. (きつれやなぎ).

SHAPE: roundish in polar view, elliptic in equatorial view. EXPANSION FOLD: 3, long. PORE: 3, situated at the middle of expansion folds; pores are sometimes inconspicuous. The EXINE tends thinner and the reticulation on the surface finer towards the edges of expansion folds. SURFACE: reticular. SIZE: 14—25  $\mu$  in diameter.

<sup>1)</sup> An exotic species investigated, *Populus pyramidalis* SALISB., is identical with this species in pollen characters.

## Aceraceae

## ACER

*Acer japonicum* THUNB. var. *typicum* GRAF v. SCHW. (はうちいかへで);  
 — *pictum* THUNB. var. *typicum* GRAF v. SCHW. subv. *eupictum* PAX  
 (いたやかへで); — *rufinerve* SIEB. et ZUCC. (うりはだかへで); — *spicatum*  
 LAM. var. *ukurunduense* MAXIM. (むがらばな); — *Tschonoskii* MAXIM. (み  
 ねかへで).

SHAPE: roundish in polar view, elliptic in equatorial view. EXPANSION  
 FOLD: 3, long. PORE: 3, situated at the middle of expansion folds;  
 pores are sometimes inconspicuous. The EXINE tends thinner towards  
 the edges of expansion folds. SURFACE: with fine wrinkles running, in  
 general, towards the poles. SIZE: 18—28  $\mu$  in diameter.

## EXPLANATION OF PLATES XI AND XII.

Magnification of figures is  $\times 580$ . In those marked with \*, the section is also  
 shown in combination.

Fig. 1. — *Taxus cuspidata*.

Fig. 2. — *Pinus densiflora*: a, lateral view\*; b, dorsal view.

Fig. 3. — *Tsuga diversifolia*: a, lateral view\*; b, dorsal view\*.

Fig. 4. — *Larix Kaempferi*.

Fig. 5. — *Cryptomeria japonica*.

Fig. 6. — *Sciadopitys verticillata*.

Fig. 7. — *Chamaecyparis obtusa*.

Fig. 8. — *Alnus japonica*: a, polar view; b, equatorial view; c, equatorial section.

Fig. 9. — *Betula Ermanii* var. *communis*: a, polar view; b, equatorial view; c, equa-  
 torial section.

Fig. 10. — *Corylus heterophylla* var. *japonica*: a, polar view; b, equatorial section.

Fig. 11. — *Carpinus carpinoides*: a, polar view; b, equatorial section.

Fig. 12. — *Celtis sinensis*: a, polar view; b, equatorial section.

Fig. 13. — *Ulmus japonica*: a, polar view; b, equatorial section.

Fig. 14. — *Zelkova serrata*: a, polar view; b, equatorial section.

Fig. 15. — *Tilia japonica*: a, polar view\*; b, equatorial view.

Fig. 16. — *Fagus crenata*: a, polar view; b, equatorial view<sup>1)</sup>; c, equatorial section.

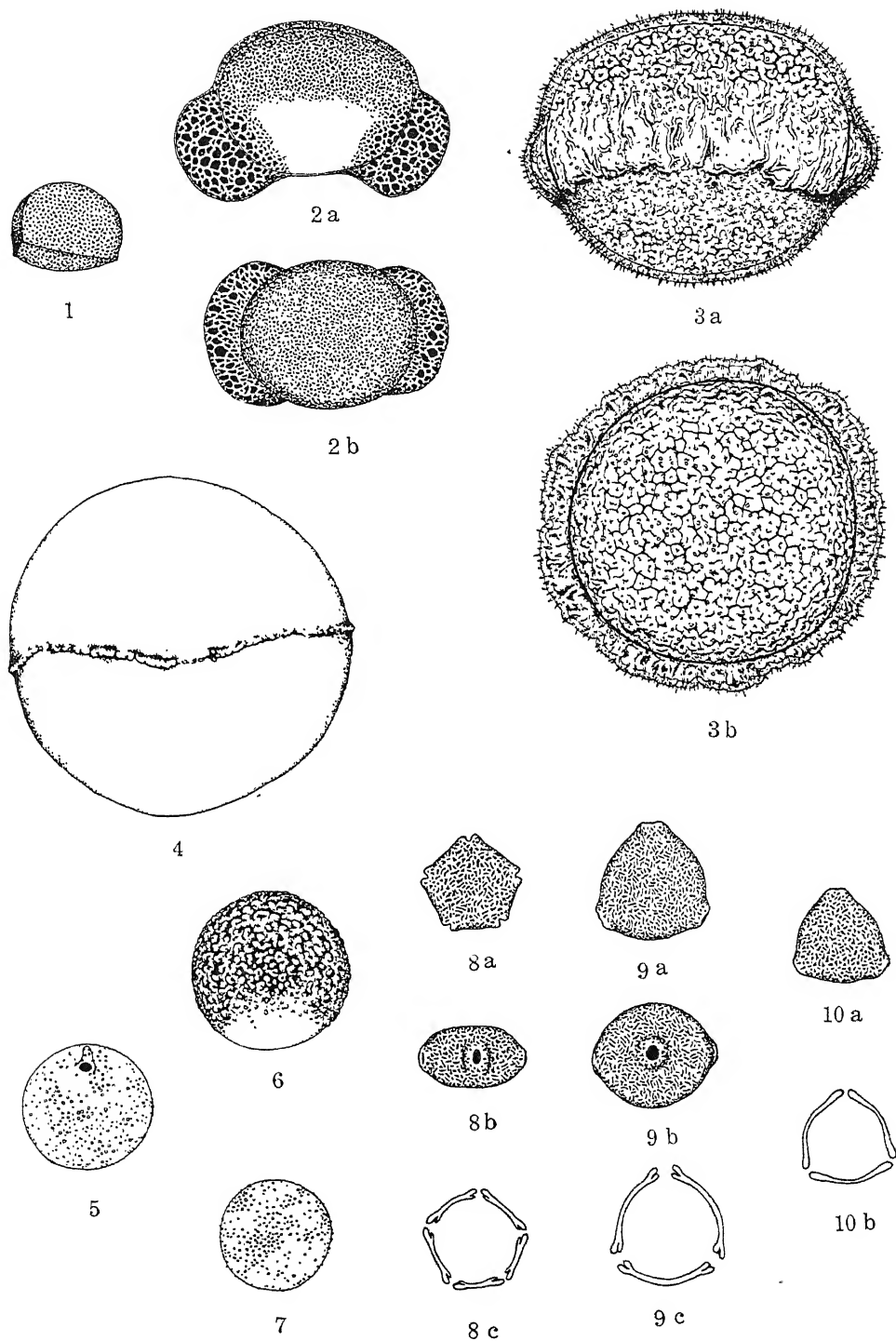
Fig. 17. — *Quercus acuta*: a, polar view\*; b, equatorial view.

Fig. 18. — *Castanea crenata*: a, polar view\*; b, equatorial view.

Fig. 19. — *Salix vulpina*: polar view\*.

Fig. 20. — *Acer Tschonoskii*: polar view\*.

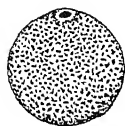
<sup>1)</sup>The hidden part of the contour of the pore is shown on purpose.







11 a



12 a



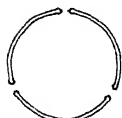
13 a



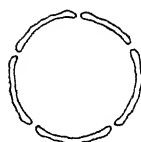
14 a



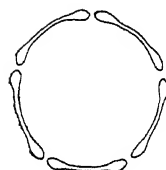
11 b



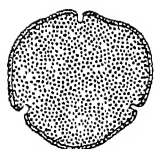
12 b



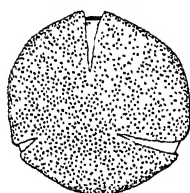
13 b



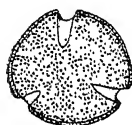
14 b



15 a



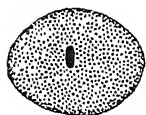
16 a



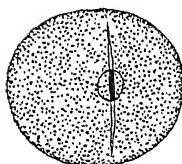
17 a



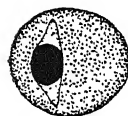
18 a



15 b



16 b



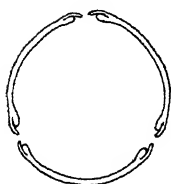
17 b



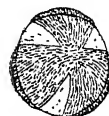
18 b



19



16 c



20



# ON THE SEASONAL AND VERTICAL DISTRIBUTION OF THE PLANKTON OF AOMORI BAY\*

By

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(With thirty-one figures)

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## 1) INTRODUCTION

The present study is the sequel to our recent paper entitled "A quantitative investigation of the plankton of Aomori Bay, as studied comparatively by pump and net collection" (1931). In the previous paper it was demonstrated that the mean quantity (from surface to bottom) of the plankton of Aomori Bay varied from 16 cc. to 529 cc. per cubic meter of water during the period 1929-1930. It was also pointed out that plankton nets of ordinary type made of No. 25 gauze is quite unsuitable for quantitative study. As regards vertical distribution, data were then insufficient to state conclusively whether it shows regular seasonal changes. Since then the work has been extended with the object of studying the quantitative change covering a longer period, and to secure data for the generalization of the law, if present, of the seasonal change of vertical distribution.

The data presented in the present investigation includes 29 observations, which were taken during May (1931) to December (1932).

Before entering the subject we wish to express grateful appreciation to The SAITO Gratitude Foundation (SAITO Hoonkai) by whom a portion of the expenses incurred in this work has been defrayed. We are also very grateful to Prof. S. HATAI under whose supervision the present work has been carried out.

## 2) METHOD

The collections were made in the same way as that employed in the previous work except that in the present case the pump only was used.

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\* Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 106.



The pump collections were made at a fixed station about 1 mile off coast where the depth measures about 31 meters. The collections and observations were made twice a month, 30 litres of water from depths of 0, 2, 4, 6, 8, 10, 15, 20, 25 and 30 meters respectively were taken at each collection. The water thus collected was filtered through No. 25 gauze, and the plankton was then fixed by adding 3% of formaline. The volume of the plankton was read after allowing it to settle for about 24 hours. The result was converted into volume per cubic meter of water. To express the representative value of each observation the volume read for each depth was averaged, thus enabling quantitative comparisons of different months to be made regardless of the vertical distribution.

All hydrographical observations were made by using the ordinary method. The dissolved oxygen, for instance, was measured by WINKLER's method, the pH by thymol blue and the water colour by FOREL's scale and so forth.

### 3) HYDROGRAPHICAL CONDITIONS

#### *1) Temperature*

According to the investigations so far made (HATAI & KOKUBO 1928, KOKUBO & TAMURA 1931, KOKUBO 1932, KOKUBO 1933) it is now certain that the thermic character of Aomori Bay is of warm temperate in its biological nature. The daily observations which have been made at the pier of the Marine Biological Station, thrice daily, shows that the highest temperature of the monthly mean (August) fluctuated from year to year between 21.93° and 25°, 37°C., the mean being 24.06°C. over a period of 5 years (1926-1930). The lowest temperature of the monthly mean (February) fluctuated between 4.20°C. and 5.94°C. over a similar period. To what extent the monthly mean deviates from the mean value of the corresponding month can be seen from the following table. Although the annual difference of temperature when corresponding months were compared is very marked, nevertheless the annual means give a very slight difference of 0.95°C. even the two extreme values were contrasted.

Coincident with the monthly change shown above the temperatures observed during 1931 and 1932 in the present investigation (Table IA) show also the maximum and minimum temperature in August and in February respectively, but are a little higher in 1931 than in 1932. This trend of water temperature is reflected similarly in the air temperature,

# PLANKTON OF AOMORI BAY

Month Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	An me
1926	5.62	5.18	5.94	7.35	11.80	15.55	20.63	22.85	22.48	16.19	12.48	8.77	12
1927	6.20	4.70	4.77	8.67	12.10	16.63	21.63	25.23	21.83	18.63	13.90	8.87	13
1928	6.67	4.97	5.80	9.33	12.53	16.40	20.43	21.93	23.63	18.90	13.20	7.90	13
1929	5.30	4.20	5.53	7.80	11.33	15.47	22.13	25.37	22.20	18.93	13.40	10.03	13
1930	5.27	6.30	7.20	9.00	12.87	16.33	20.80	24.90	23.07	18.73	13.07	8.63	13
(Mean)	5.81	5.07	5.85	8.43	12.13	16.08	21.12	24.06	22.44	18.28	13.21	8.84	13.4

that of 1931 was also higher than in 1932.

Looking through Table I A one will note that the water temper shows a vertical distribution in spite of the fact that the depth of observation station was only 31 meters. This may be due to the geographical conformation of the bay where the waves subside almost as the wind ceases, and where there is no current strong enough to bring the direct influence of oceanic currents. Consequently the surface temperature is soon influenced by the air temperature thus forming direct or indirect stratification. Though the change due to depth is not so great, its stratification can clearly be seen. In both years the indirect stratification was observed during the period from October to April, while direct stratification was found during the period from April to August.

It is always found in the case of fresh water lakes that the difference of temperature between surface and bottom water is larger in direct stratification than in indirect stratification. In Aomori Bay we found in the case of the direct stratification the maximum difference of temperature between the upper and lower layers was 4.5°C. (August 15, 1931, averaging 1.9°C., whilst the difference in indirect stratification was 0.7°C. (Jan. 19, 1931) in maximum with a mean value of 0.7°C.

It seems then that the thermic condition of Aomori Bay shows a condition similar to that of fresh water lakes. The period from Jan. to April was the period of winter stagnation. After a spring circulation occurred in between April 14, and May 1, the thermic condition passed into the summer stagnation. The summer stagnation continued till September, and the harvest circulation appeared between September and October 16. From this on till early-May the winter stagnation has been observed, and the spring circulation again appeared between

3 and April 16 (1932). After this circulation the thermic condition changed to that of the summer stagnation which ended in the autumn and manifested winter stagnation after making a harvest circulation. This cycle change seems to repeat from year to year.

## II) *Specific Gravity*

It is now well established that the specific gravity of the surface water of Aomori Bay shows remarkable seasonal changes, the lowest value being recorded in April and the highest in September. This was first reported by HATAI and KOKUBO (1928) as a result of their observations made during 1926. Approximately the same results were obtained during the succeeding four years (KOKUBO, 1933). Again we found a similar phenomena both in 1931 and in 1932 (Table I A).

Though the surface water exhibits this regular seasonal change the same does not always hold good in the water at a depth of five or more meters. Variations in the specific gravity of the deeper part seem to depend upon the atmospheric disturbance of water by the wind. In 1931, for instance, the decrease of specific gravity on May 1 and July 1 extended to the bottom and therefore caused a corresponding change in the deep layer. While in 1932 no such phenomenon was observed. The decrease of specific gravity on April 16, for instance, was restricted to the surface layer. The extreme decrease in specific gravity which was found on August 19 was a temporary change due to the heavy downpour of rain on that day.

Besides the seasonal change above mentioned there is a distinct regular vertical distribution at all seasons, showing that the specific gravity increases with the depth. Although such tendency physical law, but it may be remarkable to find it in comparatively shallow water where the difference of density between surface and bottom water is very slight.

## III) *Dissolved Oxygen*

The amount of dissolved oxygen in one litre of water ranged from 4.46 cc. to 7.42 cc. in 1931 and from 4.18 to 7.53 cc. in 1932. In both those years the lowest contents were found at a depth of about 30 meters while the highest content was found in the 5 to 10 meter layer. This distribution would suggest that in the upper layer where diatoms flourish the oxygen is produced by photosynthesis while in the bottom layer oxygen

is consumed not only by zooplankton but also by benthos. As will be seen in Table I the mean value of the 15 observations made in 1931 and 1932 shows a regular decrease of oxygen content from 10 meters downward. When considered seasonally the highest content was observed in spring (April 14, in 1931 and March 20 in 1932) and the lowest content in summer (Sept. 1 in 1931 and August 19 in 1932).

As expressed in terms of percentage saturation it ranged from 76.2 (Sept. 1) to 11.9% (July 1) in 1931 and from 72.7 (Aug. 19) to 105.3% (Mar. 20) in 1932. Looking at the figures in Table I, we are at once struck by the fact that the saturation of over 100% is observed in cases when the volume of plankton increased. In 1931, for instance, the maximum quantity of plankton (653 cc. per cubic meter) was observed on June 16, and a high saturation of oxygen was found from then on. In 1932, moreover, when the highest saturation, 103.6% (March 10), was observed the volume of plankton showed the enormous value of 1672 cc. per cubic meter of water. In the next succeeding observation made on March 20 a similar relation was recorded. The regularity of the ratio between the oxygen content and the plankton volume may be accounted for by the fact that the bulk of plankton in the above cases consisted of pelagic diatoms which produce the oxygen by photosynthesis.

With a view to ascertaining the amount of oxygen saturation due to diatoms MARSHALL and ORR (1930) made a study in Loch Striven. Their results show that during the spring increase of Diatoms (about 100,000 cells per 22 cc. of water) a saturation of over 120% was often attained at a depth from 0 to 5 meters. The maximum saturation found by them on April 6, 1928 was 138% when the Diatoms were as many as 199,000 cells per 20 cc. of water. According to YOSHIMURA and others (1930) who studied the oxygen saturation of the water of Aburatsubo Bay a high saturation of 139% was observed on Aug. 24, 1930. In our study such an extreme value has not been met with in spite of the fact that, according to the vegetation of Diatoms in Aomori Bay is very active as compared with other netitic areas of Japan (KOKUBO 1933). The lowest saturation in YOSHIMURA's observations (1930) was 73% (Nov. 18, 1928), showing a close approximation to that of the present investigation (72.7%). Recently REKESTRAW (1933) studied the oxygen saturation of the water of the Gulf of Maine in August, 1932. His results give excellent references in regard to the degree of oxygen saturation as well as its vertical distribution. According to his data the oxygen saturation of the 1 meter layer is mostly around 100%. With the increase of depth it gradually increases

reaching the maximum at about 20 meters. From this depth downward the saturation decreases reaching a saturation of 63-68% at a depth of more than 200 meters. This state of distribution is of much interest when considered in relation to the vertical distribution of plankton organisms.

In the water of an aquarium, in which the fish are kept the change of oxygen saturation show a very wide range of variation. KOKUBO (1933) found that in the normal condition of seawater aquaria the saturation decreases down to ca. 24%. Under natural conditions in the open sea a saturation of less than 50% can not be expected. A decrease of saturation under natural conditions up to 73% is thought to be due to an active biological oxydation.

#### *IV) Change of pH*

The pH of the water of Aomori Bay was studied by KOKUBO (1932). It was found that during the years 1929 and 1930 the pH of sea water ranged from 8.00 to 8.30. The annual mean was 8.21 in 1929 and 8.19 in 1930. During the period of the present investigation the range of the change of pH again varied between 8.00 and 8.30 showing values similar to 1929-1930. The annual mean was 8.21 in 1931 and 8.22 in 1932, showing a close approximation to the figures of 1929-1930.

In 1931 there were two periode of increase in the volume of phytoplankton, the first from Feb. 28 to Apr. 14 and the second on June 16. Contrasted with these increases there was a marked decrease on May 31 and on Dec. 23. In spite of such changes the pH showed almost the same value as the annual mean (8.21). The lowest value (pH 8.15) was found on May 1 when the volume of plankton approximated the annual mean, the highest value (pH 8.25) occurred on Nov. 14 when the plankton volume became about one fourth of the annual mean.

In 1932 the maximum vegetation of Diatoms was observed on the 2nd and 10th of March. No indication of this vegetation has been found in pH, although, in the latter case, only a slight rise has been observed. The highest and the lowest values of this year were respectively 8.3 and 8.00, observed on April 16 at different depths when the volume of plankton attained its minimum.

It is generally expected that there is a parallel between the pH and the volume of phytoplankton. This is because the photosynthesis of pelagic plants deprives water of  $\text{CO}_2$ , so that the increase of phytoplankton

raises the pH and vice versa. On the other hand the respiration of zooplankton decreases the pH of water by increasing the  $\text{CO}_2$  tension in water. Besides photosynthesis and respiration increase or decrease of salinity also affects the pH of water. Generally water of high salinity has a high pH and the water of low salinity a low pH. In water of low salinity the pH is highly variable due to the decrease of buffer of salts. Accordingly in water of low salinity the pH is largely affected by the photosynthesis or respiration of plankton.

Though the parallel between the pH and the volume of plankton was clearly observed in the study of the preceding two years (KOKUBO, 1932) no regular relation has been found in the present study. It may be possible, after making further investigations, to explain the course of this disturbance.

#### *V) Colour and Transparency of Sea Water*

KOKUBO's investigations during 1929-1930 revealed that the seasonal change of the colour of the water of Aomori Bay, as measured by FOREL's scale, ranged between No. 3 and No. 9. In the present investigation, however, the range was found to be more limited. In 1931 it ranged between Nos. 4 and 6 while in 1932 it ranged between No. 4 and No. 5. Comparing these values with those of the preceding two years (No. 4.4 and No. 4) it can be seen that the readings of the scale in the succeeding two years are a little lower than those of the preceding years.

If scale reading were to rise in strict relations to the increase of phytoplankton as is usually thought, the readings of 1931 should be lower than those of 1932. Because the volume of plankton, the bulk of which consisted of Diatoms, was in 1932 far greater than in 1931. But the results showed some discrepancies, the readings in 1932 being a little lower than those in 1931. From these results it will be noticed that the scale reading may be disturbed by still other factors than increase or decrease of phytoplankton, such as the contamination of water due to wind etc.

Compared with the results of the preceding two years, in which the transparency varied widely, from 2.5-24. meters, the range of change in the present investigation was found to be much less. In 1931 it ranged from 5 to 20 meters with a mean of 10. meters. In 1932 it ranged from 7.5 to 14 meters, the mean being 11.4 meters. The maximum reading in 1931 was found on Jan. 19, measuring 20 meters when the volume of

plankton decreased to the minimum value of 16 cc. When the phytoplankton reached the maximum value of 653 cc. per cubic meter of water the transparency dropped to a value of only 7 meters. The minimum value of 5 meters was observed when the plankton was moderate in quantity. Moreover relatively low values were observed even when the plankton volume was less.

Again in 1932 the highest value, 14 meters, was observed when the plankton showed its minimum increase, and the lowest value of 7.5 meters for the maximum vegetation. At the same time several cases which indicate some slight discrepancies from the general tendency of reciprocal fluctuation were found. It can, therefore be said that the reciprocal change of these two values is a regular one, but slight changes may occur due to certain other factors.

#### 4) RESULTS OF OBSERVATION

##### Observation I (Table I B., Fig. 1)

(May 1, 10.00-10.45 a.m., 1931)

The bulk of the material obtained consisted of Diatoms, represented by *Chaetoceras*. *Ch. Schiittii* was most abundant among 15 species identified in 0 meter. In the 15 meter layer were found ca. 11 species of Diatoms, but less abundance of *Ch. Schiittii* and a great abundance of zooplankton such as Dinoflagellata, Copepoda and its naupli were shown. The 30 meter layer was inhabited mainly by *Bacteriastrum* sp., *Ch. decipiens*, *Ch. Schiittii*, etc., similar as in the 15 meter layer. Thus the distribution of species differs in different depths.

TABLE I B.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m. by conversion	1.9	2.8	4.4	5.2	5.8	5.8	6.0	6.5	5.5	7.0	5.09
	63.27	93.24	146.52	173.16	193.14	193.14	199.80	216.45	183.15	233.10	169.50

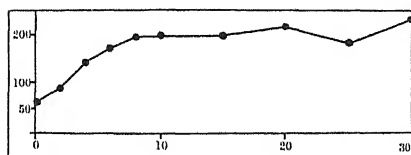
The vertical quantitative distribution is shown in Table I B and in Fig. 1. From Fig. 1 it is clear that from 0 to 8 meters the abundance of plankton increased with the depth, and from 8 meters downwards is nearly uniform.







Fig. 1.



Ordinate — volume of plankton (per cubic meter of water) in cc.

Abscissa — depth in meter.

### Observation II (Table II A., Fig. 2)

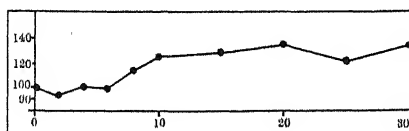
(May 13, 9.50-10.40 a.m., 1931)

The material collected from 0-20 meters showed a light cotton-like condition with a light green colour, while the material from 25-30 meters looked brownish. The species of Diatom which formed the bulk of surface material was represented by *Ch. debile*, *Ch. Schüttii*, *Ch. decipiens*, and *Bacteriastrium* sp. The fifteen and thirty meters layers exhibited almost similar conditions to the 0 meter layer, except that in the material from 30 meters a great deal of mixed debris was found. Besides the above Diatoms there were found naupli of Copepoda, several species of Dinoflagellata, 3 species of Tintinninea, larvae of Polychaeta and Gastropoda.

TABLE II A.

Depth	Surface	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Vol. of pl. (c.c)											
Actual catch per cubic m.	3.0	2.8	3.0	3.0	3.4	3.7	3.8	4.0	3.6	4.0	3.43
By conversion	99.96	93.24	99.99	93.99	113.22	123.2	126.54	133.20	119.88	133.20	114.22

Fig. 2.



The vertical quantitative distribution showed a slight tendency to increase in volume from 6 meters downwards.

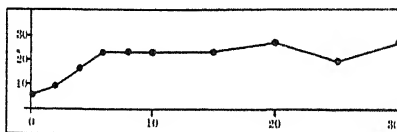
Observation III (Table III A., Fig. 3)  
(May 31, 9.40-10.20 a.m., 1931)

The quantity of plankton was very much less than in May 13. The species likewise showed a marked change, *Chaetoceras* which dominated the previous collection being found decreased (Table III A.). Replacing *Chaetoceras*, in 0 meter layer, *Thalassiothrix* and *Rhizosolenia* appeared. In the 15 meter and 30 meter layers *Coscinodiscus*, *Pleurosigma*, and *Ceratium* were found.

TABLE III A.

Depth Vol. of pl. (c.c.)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	0.2	0.3	0.5	0.7	0.7	0.7	0.7	0.8	0.6	0.8	0.60
By conversion	6.66	9.99	16.65	23.31	23.31	23.31	23.31	26.64	19.98	26.64	19.98

Fig. 3.



The quantitative vertical distribution (Fig. 3) showed rapid increase from 0 to 6 meters but from 6 meters downwards, the distribution was almost equal.

The quantitative vertical distribution (Fig. 3) shows that from 0 to 6 meters the plankton relatively rapidly increase, from 6 meters downwards, however, the distribution was almost equal. This mean value found was smallest throughout the whole period of the present investigation.

Observation IV (Table IV A., Fig. 4)  
(June 15, 9.20-10.00 a.m., 1931)

Contrary to the marked decrease which was found in the previous observation the present collection showed a remarkable increase. The species appeared have also been found altered. The materials were represented by Diatoms, especially by *Ch. Schüttii*, *Rhizosolenia hebetata*, *Rhizosolenia hebetata*, *Thalassiothrix nitzschoides* being next in abundance.

TABLE IV A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	30.0	17.0	20.0	26.0	22.5	30.0	24.5	15.5	7.5	4.0	19.70
By conversion	999.99	566.10	666.60	865.80	749.25	999.99	815.85	516.15	239.75	133.20	652.55

Fig. 4.



The quantitative vertical distribution showed a distinct difference from that of the preceding experiments. The distribution between 0 and 10 meters was very irregular, but from 10 meter downwards a tendency of rapid decrease was observed.

## Observation V. (Table V A., Fig. 5)

(July 1, 9.30-10.15 a.m., 1931)

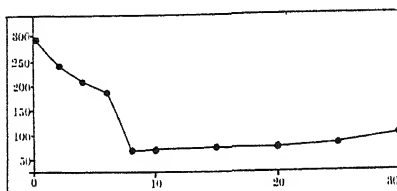
The plankton again showed a decrease. The species of Diatoms almost the same as in the previous observation, *Ch. Schiittii*, *Ch. decipiens*, and *Rhizosolenia hebetata* being found through the whole depth. *Chaetoceras* which showed the greatest abundance showed a marked decrease. *Nitzschia seriata*, *Rhizosolenia alata*, and *Pleurosigma sp.* were much restricted

TABLE V A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	8.6	7.2	6.2	5.5	2.0	2.1	2.2	2.2	2.5	3.1	4.16
By conversion	286.38	239.76	206.46	183.15	66.66	69.93	73.26	73.26	83.25	103.23	138.53

to the deep layer. Zooplankton such as *Peribinium*, *Ceratium*, Copepoda, larvae of Copepoda and Polychaeta, and *Fritilaria* were also found, but in a very low abundance.

Fig. 5.



## Observation VI (Table VI A., Fig. 6)

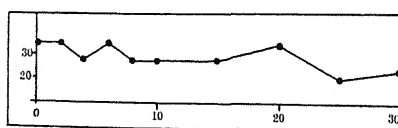
(July 15, 9.30-10.10 a.m., 1931)

The quantity of plankton is less than in the previous collection. The species appeared showed no remarkable change as compared with the previous case. The leading species were *Rhizosolenia alata*, *Rhizosolenia bedetata*, *Thalassiothrix nitzschioides*, etc. The species of zooplankton were also much the same as in the previous observation.

TABLE VI A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.0	1.0	0.8	1.0	0.8	0.8	0.8	1.0	0.6	0.7	0.85
By conversion	33.33	33.33	26.64	33.33	26.64	26.61	26.61	33.33	19.98	23.31	28.32

Fig. 6.



## Observation VII (Table VII A., Fig. 7)

(August 1, 10.00-10.50 a.m., 1931)

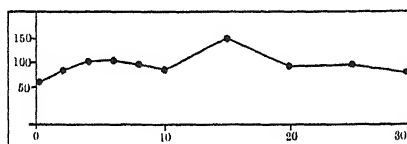
The plankton showed an increase since the previous collection. The Diatoms invariably predominated the bulk of plankton. The leading species were *Chaetoceras sociale*, *Thalassiothrix nitzschioides*, etc. *Ch.*

*decipiens*, *Rhizosolenia Stolterfothii*, *Hemiaulus* sp., *Coscinodiscus* sp. etc. were also found. Among the zooplankton *Goniaulax*, *Ceratium*, *Peridinium*, nauplius of Copepoda, *Tintinnus*, *Undella* etc. were common.

TABLE VII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.8	2.5	3.0	3.1	2.9	2.6	4.5	2.8	2.9	2.4	2.85
By conversion	59.94	83.25	99.99	103.23	96.57	86.58	149.85	93.24	96.57	79.92	94.91

Fig. 7.



Observation VIII (Table VIII A., Fig. 8)

(August 15, 9.30-10.15 a.m., 1931)

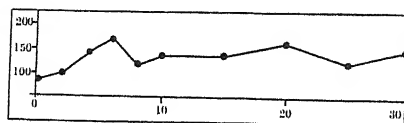
The plankton increased slightly as compared with the previous observation. *Chaetoceras sociale* was the first in abundance, *Thalassiothrix* being the next. From 15 meters downwards were mainly crowded by *Ch. didymum*, *Ch. debile*, *Ch. Schüttii*, *Dactylosolen tenuis*, *Thalassiothrix nitzschoides*, etc. Zooplankton were comparatively scanty and were represented by *Ceratium*, *Peridinium*, *Goniaulax*, *Tintinnopsis*, *Oikopleura*, *Fritilaria*, *Copepoda*, etc.

TABLE VIII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	2.5	3.0	4.2	5.0	3.5	4.0	4.0	4.8	3.6	4.5	3.91
By conversion	83.33	99.99	139.99	166.65	116.65	133.32	133.32	159.98	119.99	149.99	130.32

From 0 to 6 meters an increasing tendency was shown, but from this downwards no definite tendency has been observed.

Fig. 8.



Observation IX (Table IX A., Fig. 9)

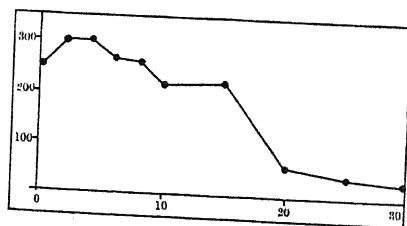
(September 1, 9.00-10.15 a.m., 1931)

In this collection the specimens obtained from the upper showed a cotton-like appearance while that from bottom layer showed a dense powder-like condition. The former mainly consisted of several species of *Chaetoceras* i.e. *Ch. debile*, *Ch. sociale*, *Ch. decipiens*, *Ch. Schüttii*, *Ch. didymum*, etc. The latter consisted of spine-less diatoms e.g. *Thalassiosira*, *Rhizosolenia*, *Thalassiothrix*, *Coscinodiscus*, *Climacodium*, *Hemiaulus*, *Navicula*, etc. Zooplankton consisted of *Ceratium*, *Peridinium*, *Tintinnus*, *Parafavella*, Copepoda, *Oikopleura*, larvae of Copepoda and Polychaeta.

TABLE IX A.

Depth	Surface	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Vol. of pl. (c.c)											
Actual catch per cubic m.	7.5	9.0	9.0	8.0	7.8	6.5	3.6	1.7	1.2	0.9	5.52
By conversion	249.75	299.70	239.70	266.40	259.74	216.45	119.88	56.61	39.96	29.97	183.82

Fig. 9.



A sudden decrease was found between 15 and 30 meters.

Observation X (Table X A., Fig. 10)

(September 16, p. 30-10.20 a.m., 1931)

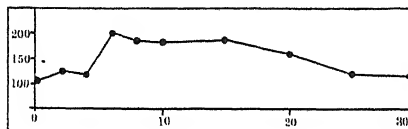
*Ch. debile* and *Ch. sociale* which were the leading species in the previous collection have quite disappeared though other species remained

unchanged. The species which showed the highest abundance were *Ch. Schiittii* and *Ch. didymum*, followed by *Rhizosolenia* and *Dactyliosolen*. Of the zooplankton collected *Goniaulax* inhabited the layer from the surface to middle layer, while *Peridinium*, Copepoda, and *Oikopleura* were much more plentiful in the lower layer than in the upper layer.

TABLE X A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	3.2	3.6	3.5	6.0	5.5	5.4	5.5	4.6	3.5	3.5	4.43
By conversion	106.56	119.88	116.55	199.80	183.15	179.82	183.15	153.18	116.55	116.55	147.42

Fig. 10.



From surface to 6 meters a rapid increase was observed followed by a gradual decrease down to 30 meters.

## Observation XI (Table XI A., Fig. XI)

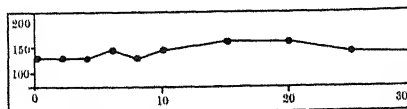
(October 9.10-9.45 a m., 1931)

The bulk of the present collection was represented by *Bacteriastrium* which was less abundant in the previous collection. Besides the above species *Thalassiothrix*, *Chaetoceras*, and *Asterionella* were observed.

TABLE XI A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	4.0	4.0	4.0	4.5	4.0	4.5	5.0	5.0	4.5	4.5	4.40
By conversion	133.20	133.20	133.20	149.85	133.20	149.85	166.50	166.50	149.85	149.85	146.52

Fig. 11.





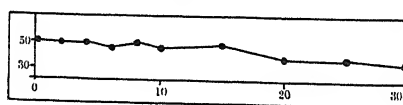
Observation XII (Table XII A., Fig. 12)  
(November 14, 9.40-10.30 a.m., 1931)

Since the preceding collection the quantity of plankton showed a prominent decrease. *Bacteriastrium* which showed a high abundance in the previous collection became fewer. *Thalassiothrix nitzschoides*, *Biddulphia sinensis*, and *Bacteriastrium* sp. were the main representatives of the Diatoms.

TABLE XII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.5	1.5	1.5	1.4	1.5	1.4	1.5	1.2	1.2	1.1	1.38
By conversion	49.95	49.95	49.95	46.62	49.95	46.62	49.95	39.96	39.96	36.63	46.29

Fig. 12.



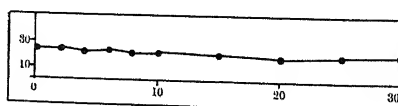
Observation XIII (Table XIII A., Fig. 13)  
(December 23, 9.40-10.30 a.m., 1931)

The present collection showed a further decrease of plankton. But the species that appeared were almost the same as those of the previous collection, though a minor difference was found in the point that *Coscinodiscus* which was seen only in the deep layer was found distributed throughout the whole depth. The vertical distribution was very equal.

TABLE XIII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	0.75	0.75	0.7	0.75	0.70	0.70	0.70	0.60	0.65	0.75	0.72
By conversion	24.98	24.98	23.31	24.98	23.31	23.31	23.31	19.98	21.95	24.98	23.51

Fig. 13.



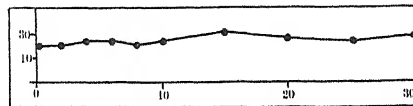
Observation XIV (Table XIV A., Fig. 14)  
(January 21, 9.40-10.20 a.m., 1932)

The present collection closely resembles that of the previous collection. Though a marked difference was noticed in the point that *Merosira Borreri* which was absent in the previous case appeared and was distributed abundantly throughout the whole depth.

TABLE XIV A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	0.6	0.6	0.7	0.7	0.6	0.7	0.9	0.75	0.7	0.8	0.71
By conversion	19.98	19.98	23.31	23.31	19.98	23.31	29.97	21.98	23.31	26.61	23.48

Fig. 14.



In the present collection, too, the vertical distribution was very uniform.

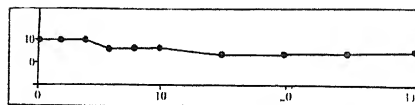
Observation XV (Table XV A., Fig. 15)  
(February 1, 9.30-10.10 a.m., 1932)

The plankton showed a slight increase as compared with the former collection. The specimen obtained from 0 to 15 meters was light and cotton-like in appearance, while that from the deeper layers were dense and powder-like. Unlike the previous collection *Chaetoceras sociale* showed a dominance. The abundance of each species was, in order, as follows; *Biddulphia sinensis*, *Thalassiothrix nitzschoides*, *Coscinodiscus* sp., *Thalassiosira* sp., *Ditylium Brightwellii*, etc. Of the zooplankton *Ceratium furca*, *C. fursus*, *Oikopleura* sp. and Copepoda have been noted.

TABLE XV A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.2	1.2	1.2	1.0	1.0	1.0	0.8	0.8	0.8	0.8	0.98
By conversion	39.96	39.96	39.96	33.33	33.33	33.33	26.64	26.64	26.64	26.64	32.63

Fig. 15.



As in the former collection the vertical distribution was very even throughout the whole depth.

### Observation XVI (Table XVI A., Fig. 16)

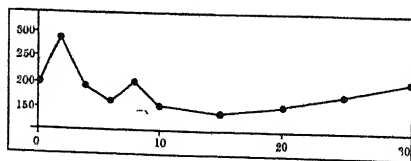
(February 15, 9.30-10.10 a.m., 1932)

The increase of plankton has been pronouncedly noticed, probably suggesting the start of the spring flourishing of Diatoms. *Ch. sociale* and *Ch. debile* dominated throughout the whole depth. In place of *Biddulphia*, *Corethron*, and *Ditylimum* which showed a moderate abundance in the previous collection *Coscinodiscus Janischii*, *Asterionella japonica* etc. were found.

TABLE XVI A.

Depth Vol of pl. (c.c.)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	5.9	8.8	5.8	5.0	6.0	4.6	4.2	1.5	5.2	6.0	5.60
By conversion	196.47	293.04	193.14	166.50	199.80	153.18	139.86	149.85	173.16	199.80	186.48

Fig. 16.



The maximum was found at 2 meters and the minimum at 15 meters. The distribution became irregular as compared with the previous case.

### Observation XVII (Table XVII A., Fig. 17)

(March 10, 9.30-10.10 a.m., 1932)

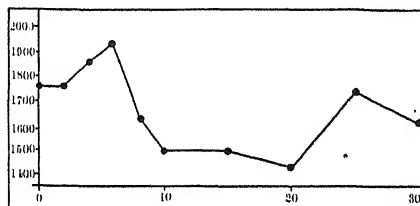
The vegetation of Diatoms has now reached the maximum flourishing. The specimens showed a light cotton-like appearance. The bulk of the

species consisted of *Ch. sociale* and *Ch. debile*, other species e.g. *Coscinodiscus Janischii* and *Nitzschia seriata*, etc. being next in abundance. As has been usually so zooplankton such as *Ceratium*, Copepoda, *Oikopleura* were found, but playing only a minor part.

TABLE XVII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	53.0	53.0	56.0	58.0	49.0	45.0	45.0	43.0	52.0	48.0	50.20
By conversion	1764.50	1764.50	1864.80	1931.40	1631.70	1493.50	1493.50	1431.50	1731.60	1593.40	1671.66

Fig. 17.



Very striking maximum and minimum have first been found since 1929. The quantitative vertical distribution was very irregular as the table shows.

## Observation XVIII (Table XVIII A., Fig. 18)

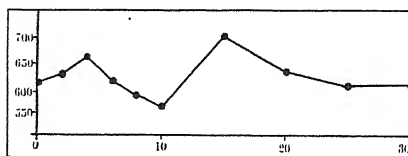
(March 20, 8.45-9.30 a.m., 1932)

The present observation was made 10 days after the previous collection, with the result that the quantity of plankton decreased to a great extent within these 10 days. This seems to indicate the decline of spring maximum of Diatoms. As in the previous observation the dominating species were *Ch. sociale* and *Ch. debile*, with a decrease of *Coscinodiscus Janischii*.

TABLE XVIII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	18.5	20.5	20.0	18.5	17.5	17.0	21.0	19.0	18.0	18.0	18.80
By conversion	616.05	632.65	666.66	616.05	582.75	566.10	699.30	632.70	599.40	599.40	626.10

Fig. 18.



The total collection decreased less than a half of the previous collection. The vertical distribution was irregular.

#### Observation XIX (Table XIX A., Fig. 19)

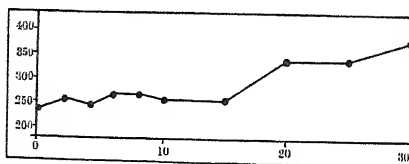
(April 3, 9.10-9.50 a.m., 1932)

A further decrease of the quantity of plankton was observed since the marked decrease observed in the previous collection. The species appeared was almost the same as in the previous observation.

TABLE XIX A.

Depth Vol. of pl. (c.c.)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	7.2	7.7	7.4	8.0	8.0	7.7	7.6	10.0	10.0	11.2	8.47
By conversion	239.80	256.50	246.40	266.40	266.40	256.50	253.10	333.00	333.00	373.00	282.50

Fig. 19.



The vertical distribution was such that the volume from surface to 15 meters was almost equal. From this downwards, however, a decreasing tendency has been observed.

#### Observation XX (Table XX A., Fig. 20)

(April 16, 9.40-10.45 a.m., 1932)

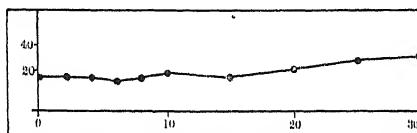
The volume of plankton which was steadily decreasing since March 20 reached a final decrease, probably suggesting a forthcoming increase.

Of the species of Diatoms that appeared, *Coscinodiscus Janischii* showed a relatively high abundance, followed by *Pleurosigma affine* and *Coscinodiscus*. The zooplankton associated with these Diatoms were represented by *Ceratium fusus*, *C. tripos*, Copepoda, larvae of Copepoda and *Limacina*. *Chaetoceras* which dominated the Diatoms of the previous collection was found to have almost disappeared.

TABLE XX A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	0.5	0.5	0.5	0.4	0.5	0.6	0.5	0.7	0.9	1.0	0.61
By conversion	16.65	16.65	16.65	13.32	16.65	19.98	16.65	23.31	29.97	33.33	20.31

Fig. 20.



The vertical distribution was almost equal from the surface to bottom layer. A slight tendency to increase the volume toward the bottom might possibly be due to the debris which also increases with the depth.

## Observation XXI (Table XXI A., Fig. 21)

(May 1, 9.40-10.20 a.m., 1932)

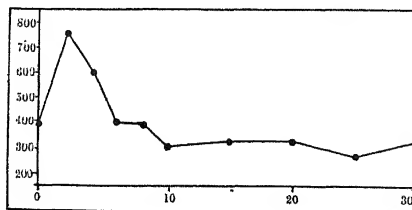
The volume of plankton now began to increase considerably, probably being the secondary vegetation which is often found after an unusual decrease. The leading species was *Bacteriastrium sp.* accompanied by *Eucampia*, *Chaetoceras*, *Thalassithrix*, *Coscinodiscus*, and *Pleurosigma*. Zooplankton such as *Ceratium bucephalum*, *C. fusus*, *C. furca*, *Peridinium spp*, *Dinophysis*, *Oikopleura* and Copepoda were also found. The remarkable difference from the previous collection was the replacement of the leadership of *Coscinodiscus* by *Bacteriastrium*.

The mode of vertical distribution was very striking. From surface to 2 meter layer the volume increased very rapidly, from this to 10 meters, however, plankton showed a striking decrease, and from 10 meters to 30

TABLE XXI A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	11.7	22.5	18.0	12.0	11.5	8.8	9.4	9.5	7.6	9.4	12.04
By conversion	390.00	750.0	600.0	400.0	383.3	293.3	313.3	316.6	253.3	313.3	401.31

Fig. 21.



meters the distribution was almost equal. MARSHALL and ORR (1930) stated that the Diatom first increases at the surface reaching the 5 fathom layer a week later. From their suggestion it may be surmised that this sudden increase in 2 m. might indicate an increase in the deeper layer later.

### Observation XXII (Table XXII A., Fig. 22)

(June 15, 9.10-9.55 a.m., 1932)

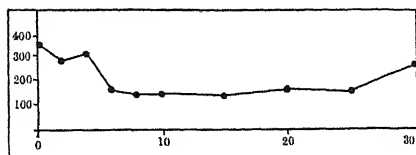
Since previous collections a decreasing tendency has been observed. The community of the plankton was almost the same as in the previous collection, *Bacteriastrum* being the leading species.

TABLE XXII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	11.0	8.8	9.5	5.2	4.6	4.6	4.3	4.8	4.5	7.3	6.56
By conversion	366.66	293.33	316.66	173.33	153.33	153.33	143.33	160.00	150.00	243.33	215.33

The vertical distribution was in such a state that the maximum of 360 cc. was found at the surface and it gradually decreased down to 153 cc. in 8 meters. From 8 to the 25 meters the distribution was much uniform

Fig. 22.



though an increase was observed at the 30 meters layer. Consequently, the abundance was high at the surface and low at the bottom layer.

### Observation XXIII (Table XXIII A., Fig. 23)

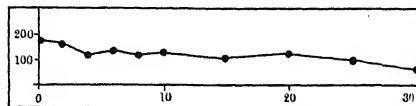
(July 16, 8.40-9.20 a.m., 1932)

A decreasing tendency became more evident since the collection on May 1. The diatoms invariably predominating the bulk of the specimens. But it differed from the previous collection in the point that *Bacteriastrium* which dominated the previous catch decreased and the specimens were represented by *Rhizosolenia hebetata*, *Nitzschia seriata*, *Chaetoceras Schüttii*, *Thalassiothrix nitzschoides*, etc. Among zooplankton *Pyrophacus* and *Goniaulax* were noted.

TABLE XXIII A.

Depth	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Vol. of pl. (c.c)											
Actual catch per cubic m.	5.4	5.0	3.5	4.0	3.5	3.7	3.0	3.5	2.5	1.4	3.55
By conversion	180.00	166.66	116.66	133.33	116.66	123.33	100.00	116.66	83.33	46.66	118.32

Fig. 23.



### Observation XXIV (Table XXIV A., Fig. 24)

(August 1. 9.50-10.30 a.m., 1932)

Compared with the previous observation an increase of plankton especially due to the vegetation of *Chaetoceras* has been observed. Dominating species are; *Ch. debile* accompanying *Ch. decipiens*, *Ch. Schüttii*,

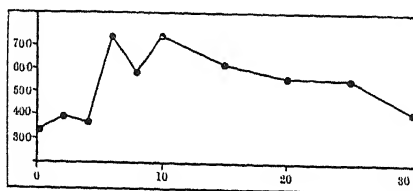


*Ch. didymum*, *Bacteriastrium* sp. *Rhizosolenia alata*, *Thalassiothrix nitzschoides* etc. Zooplankton were found mixed with Diatoms, *Parafavella gigantia*, larvae of Lamellibranchs etc.

TABLE XXIV A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	10.0	11.5	10.8	21.0	16.8	21.0	17.5	15.8	15.5	11.5	15.19
By conversion	333.33	383.33	360.00	700.00	560.00	700.00	583.33	526.66	516.66	383.33	456.40

Fig. 24.



The vertical distribution showed an abrupt increase of plankton from 4 to 10 meters followed by a slight decrease with the depth.

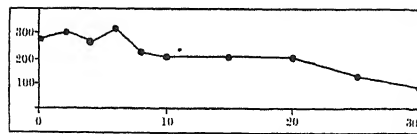
Observation XXV (Table XXV A., Fig. 25)  
(August 19, 10.00-10.50 a.m., 1932)

The plankton which showed a great increase in the previous collection decreased in the present observation. The material obtained by the present collection showed a light cotton-like appearance. Besides *Ch. debile* and *Ch. Schüttii*, which dominated *Ch. decipiens* and *Bacteriastrium* sp. were also found. The materials obtained from the bottom layer were mixed with debris.

TABLE XXV A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	8.2	9.0	8.0	9.5	6.6	6.0	6.0	5.8	3.5	2.0	6.46
By conversion	273.33	300.00	266.66	316.66	220.00	200.00	200.00	193.33	116.66	66.66	216.00

Fig. 25.



Between this (Obs. No. XXV) and the following (Obs. No. XXVI) collections two separate additional collections were made on October 15th and 31st. In these collections 32 litres of water were taken continuously from 30 meters up to 0 meter layer. These results are inserted in Table XXXI and Fig. 31.

#### Observation XXVI (Table XXVI A., Fig. 26)

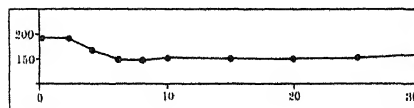
(October 31, 8.50-9.40 a.m., 1932)

The quantity of plankton showed no remarkable change as compared with the previous observation. Representative species were *Ch. Schiittii*, *Ch. debile*, *Ch. decipiens*, and *Ch. contortum*. In this collection it was notable that the species of *Chaetoceras* crowded the upper layer and *Thalassiothrix Frauenfeldii*, *Asterionella japonica* inhabited the middle layer, while *Nitzschia seriata* and *Biddulphia sinensis* were found at the bottom layer.

TABLE XXVI A.

Depth Vol. of pl. (c.c.)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	5.8	5.8	5.0	4.3	4.4	4.5	4.3	4.3	4.4	4.5	4.73
By conversion	193.33	193.33	166.66	143.33	146.66	150.00	143.33	143.33	146.66	150.00	157.66

Fig. 26.



The vertical distribution was uniform showing only a slight tendency of decrease with the depth.

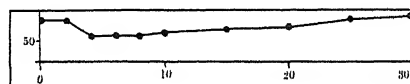
Observation XXVII (Table XXVII A., Fig. 27)  
(November 14, 10.10-10.55 a.m., 1932)

In comparison to the result of the previous collection the volume of plankton showed a distinct decrease. *Chaetoceras* which dominated the specimens of the previous collection was replaced by *Rhizosolenia* and *Thalassiothrix*. *Thalassiothrix* showed the highest abundance at the 15 meter layer.

TABLE XXVII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	2.4	2.4	1.5	1.5	1.6	1.6	1.8	1.7	2.3	2.4	1.92
By conversion	80.00	80.00	50.00	50.00	53.33	53.33	60.00	56.66	76.66	80.00	63.99

Fig. 27.



Observation XXVIII (Table XXVIII A., Fig. 28)  
(December 4, 9.10-9.55 a.m., 1932)

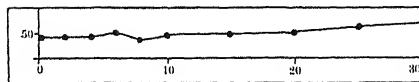
The quantity of plankton became less than in the previous observation. The specimens chiefly consisted of *Thalassiothrix nitzschoides*, *Ch. debile*, *Ch. Schüttii*, *Ditylium Brightwellii* and *Rhizosolenia setigera*. Of the zooplankton *Peridinium*, *Ceratium*, Copepoda, *Sagitta*, *Oikopleura*, larvae of Copepoda and *Pilidium* were commonly found.

TABLE XXVIII A.

Depth Vol. of pl. (c.c)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.2	1.2	1.2	1.3	1.1	1.2	1.2	1.3	1.3	1.6	1.26
By conversion	40.00	40.00	40.00	43.33	36.66	40.00	40.00	43.33	43.33	53.33	41.99

The volume of plankton showed but little difference according to depth, resulting in uniform distribution.

Fig. 28.



Observation XXIX (Table XXIX A., Fig. 29)

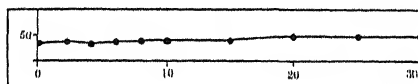
(December 19, 9.10-9.50, a.m., 1932)

Compared with the former collection the quantity of plankton showed a further decrease probably reaching the minimum of the year. The species appeared showed but little difference as compared with the previous case, chiefly consisting of *Thalassiothrix*, *Chaetoceras*, *Rhizosolenia*, *Bacteriastrium* etc. As is usually the case the plankton showed a very uniform distribution when it showed a decrease.

TABLE XXIX A.

Depth Vol. of pl. (c.c.)	Sur- face	2m	4m	6m	8m	10m	15m	20m	25m	30m	Mean
Actual catch per cubic m.	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0
By conversion	33.33	33.33	30.00	33.33	33.33	33.33	33.33	36.66	33.33	33.33	33.33

Fig. 29.



## 5) GENERAL REMARKS

## 1) Seasonal Change in Quantity

In studying the seasonal quantitative change of plankton primary attention has been paid to the seasonal flourishing of the Diatoms. This is because the quantitative change of the volume of plankton in Aomori Bay is due chiefly to the increase or decrease of Diatoms. In this regard it was reported in the previous work (KOKUBO and TAMURA, 1931) that in Aomori Bay prominent vegetation was found twice a year in September and in March, from this fact just mentioned one may assume that there are two periods, flourishing with diatoms a year viz, a vernal and an autumnal increase.

TABLE XXXI.

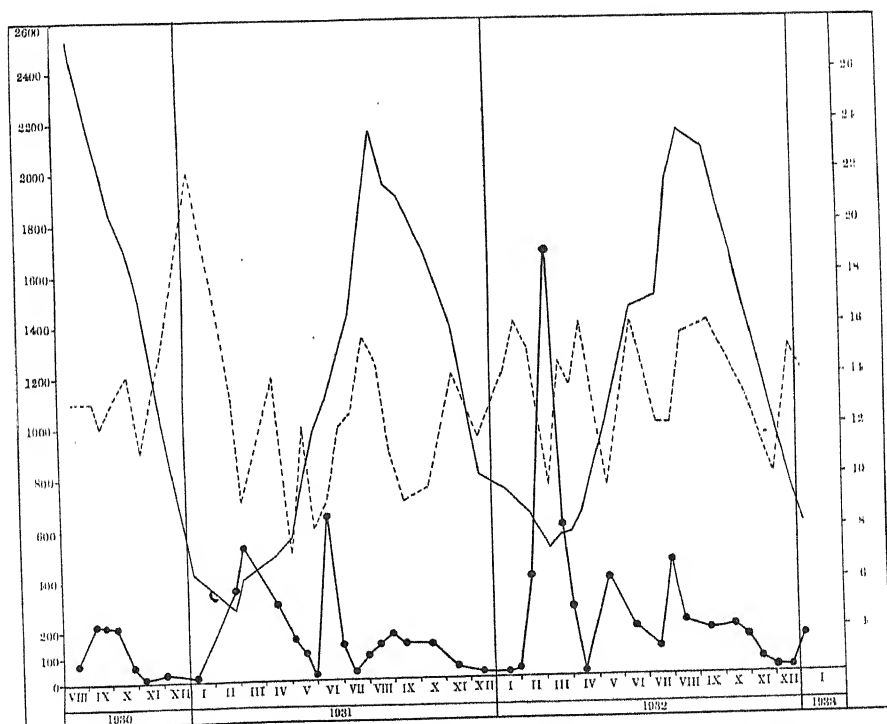
Date 1931-1932			No. Obs.	Vol. of Plankton cc in m <sup>3</sup>	
				Each coll.	Monthly ave.
1931	Jan.	19	(IX)	16.0	16.0
	Feb.	28	(X)	362.7	362.7
	Mar.	11	(XI)	529.4	529.4
	Apr.	14	(XII)	311.3	311.3
	May	1	I	169.5	
		13	II	114.2	101.2
		31	III	20.0	
	June	15	IV	652.6	652.6
	July	1	V	138.5	
		15	VI	28.3	83.4
	Aug.	1	VII	94.9	
		15	VIII	130.3	112.6
	Sept.	1	IX	183.8	
		16	X	147.4	165.6
	Oct.	16	XI	146.5	146.5
	Nov.	14	XII	46.3	46.3
	Dec.	23	XIII	23.5	23.5
(mean)				183.2	
1932	Jan.	21	XIV	23.5	23.5
	Feb.	1	XV	32.6	
		15	XVI	186.5	109.6
	Mar.	10	XVII	1671.7	
		20	XVIII	626.1	1148.9
	Apr.	3	XIX	282.5	
		16	XX	20.3	151.4
	May	17	XXI	401.3	401.3
	June	15	XXII	215.3	215.3
	July	16	XXIII	118.3	118.3
	Aug.	1	XXIV	456.4	
		19	XXV	216.0	336.2
	Sept.	16	*	187.8	187.8
	Oct.	15	*	200.0	
		31	XX V	157.7	178.8
	Nov.	14	XXVII	64.0	64.0
	Dec.	4	XXVIII	42.0	
		19	XXIX	33.3	37.7
	(mean)				274.1

\* Collections made by taking water continuously from 30 meters up to 0 meter.

The extension of the work, however, shows that such is not always the case. As is evident from Table XXXI and Fig. 30 in 1931 three periods of increase were observed in March, in June, and in September. Of these three increases the highest abundance (653 cc.) was seen in June, that of March (529 cc.) being rather less, while that of September was evident though least in abundance. The first increase in March is, presumably the annual vernal flourishing, whilst that of July may be regarded as a temporary vegetation due to some occasional causes. The increase in September may represent the autumnal flourishing though it is not very pronounced.

On March 10th, 1932 a remarkable vernal flourishing was observed,

Fig. 30.



Ordinate (left side)—volume of plankton in cc (per cubic meter of water),  
and transparency in cm.

Ordinate (right side) temperature of sea water, C°.

Abscissa—year and month.

Thick line connecting black point represents volume of plankton.

Broken line represents the transparency.

Continued line represents the temperature.

which far exceeded that of any previous observation. This vernal increase, however, suddenly subsided reaching, by March the minimum for the year. During the remainder of the year two periods of moderate increase were observed, one in May and another in August. Both of these increases with the exception of the autumnal one have been of a merely temporary character. Hence it will be seen from Fig. 30 that the mode of the seasonal change differs highly in both years.

KOKUBO (1932) who studied the seasonal changes of Diatoms at the same station by the counting method found that the mode of seasonal change varies from year to year. Moreover, his later work (1933) in which the plankton of 1931 was dealt with shows close coincidence with the present investigation in that, in March and July pronounced increases were found. As may be seen from Table XXXI the annual mean was 183.2 cc. in 1931 and 274 cc. in 1932, indicating that the latter is about 1.5 times of the former. KOKUBO (1932) estimates, by the counting method, that the plankton in 1930 was 2.47 times as much as that of 1929, likewise indicating the fluctuation of abundance according to year.

Summing up our present and the previous investigation (1931) it is concluded that in Aomori Bay the vernal increase of Diatoms is very marked and can regularly be observed every year during the period from late-February to April. Following the vernal increase one or two periods of temporary increase may occur between then and August. The harvest increase is also probable though the abundance seems to be low as compared with the vernal increase. Whether the harvest increase occurs markedly or not seems to be due to the autumnal hydrographical conditions which change from year to year. JOHNSTONE and others (1924) also indicated in the vernal increase a greater abundance of Diatoms than that in the harvest increase.

During the period from August (1930) to December (1932) the monthly mean volume of plankton per cubic meter of water ranged from the minimum of 16 cc. (Jan. 19, 1931) to the maximum of 1148.9 cc. (March 10, 1932). The change of the mean of each collection during the same period varied between 16 cc. (Jan. 19, 1931) and 1671.7 cc. (March 10, 1932). The maximum and minimum show a wide range of variation when those taken from every depth and through the whole period (1930-1932) were compared. The minimum of 10 cc. was observed on January 19th, 1931 at the 0 meter layer and the maximum of 1931 cc. was observed on March 10th, 1932 at the 6 meter depth. Assuming that the mean (1672 cc.) of observation No. XVII (March 10, 1932) at the station of observation

is representative of Aomori Bay and that the mean depth of the whole area is 40 meters, the production of plankton per square meter is estimated to be about 6.7 cubic meter. According to APSTEIN (1906) the maximum abundance of the plankton of the North Sea on November 12th at a depth of 22 meters was estimated to be 1240 cc. per square meter. Comparing this value with that of Aomori Bay there appears to be a striking difference in the yield of plankton between these two seas.

## 2) Change of Vertical Distribution

As the mode and change of the vertical distribution were the main object, the data has been thoroughly examined from this angle. In the previous paper (KOKUBO and TAMURA, 1931) have shown how, in summer, the Diatoms crowd the upper layer, and how with the advance of the season, the distribution gradually became equal from surface to bottom. The probability that distribution becomes irregular during the vernal flourishing drew our attention. Continued observations have revealed that some of the above contentions were not entirely justified and that the data obtained thereafter afforded explanation of the changes of vertical distribution.

Looking through Fig. 1-29 the mode of the vertical distribution can be classified into four types, i. e. (1) upper crowding, (2) uniform distribution, (3) irregular distribution, (4) lower crowding. The following table is based on the data obtained from 29 observations.

[illegible]



From the above table it will be noted that among the four types of distribution Type I appears most frequently (45%), Type II being next, showing a frequency of 35%. Type III and IV appear only at intervals. Consequently it follows that the common type of distribution in Aomori Bay is Type II, the succeeding one being Type I.

Considering the change of vertical distribution seasonally it is significant that when the Diatoms increase their distribution became dense in the upper layer (Obs. No. I-V) but with a decrease (Obs. No. 6-8) it became uniform. Again, when the Diatoms increased Type I appeared (Obs. No. IX). After this, and until February 1932, a decrease set in and distribution accordingly became uniform for this period (Obs. No. X-XV). During March of 1932 the Diatoms increased abnormally resulting in irregular distribution (Obs. No. XVII, XVIII). Thereafter distribution Type IV (lower crowding) was observed (Obs. No. XIX). From this until December (Obs. No. XXXIII) similar changes were repeated.

From what has just been mentioned it can be concluded that, in all probability, distribution Type I (upper crowding) appears as the result of a normal increase of Diatoms. Distribution Type II, i. e. equal distribution, occurs when the Diatoms decrease. When an abnormal increase has occurred distribution becomes at first irregular (Type III) and then lower crowding distribution (Type IV) follows. This explanation makes necessary a partial modification of the statement contained in our previous paper (1931) in which "upper crowding" distribution occurs in summer only. The present results suggest that the "upper crowding" distribution observed during summer was a normal result of the increase of Diatoms and the equal distribution (Obs. No. VI-IX of previous work) observed later was the result of a decrease of Diatoms.

The fact that the distribution of "upper crowding" appears with the increase of Diatoms may be due to their characteristic mode of vegetation. According to MARSHALL and ORR (1930) the increase of Diatoms in Loch Striven takes place at first at the surface and sinks gradually into deep water, so that the maximum at 5 fathoms occurred a week after the maximum at the surface has appeared. If this be the case in Aomori Bay it is plain that with the increase of Diatoms the upper layer should be more densely crowded than in the lower. Irregular or lower crowding distribution may be due to the gradual fall of the swarming flock of declined Diatoms.

Thus far the mode and change of vertical distribution has been described. There is, however, another tendency which deserves comment.

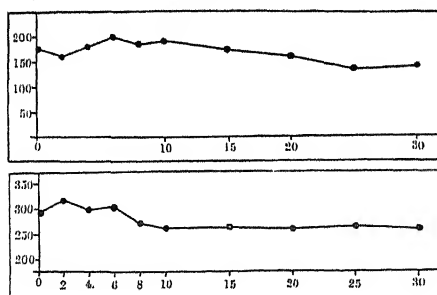
Glancing through Figs. 1-29 it will be noted that when the plankton showed an apparent increase, e. g. over 150 cc., the quantity at surface is mostly smaller than at 2 meters. In ten out of twelve cases, in which the plankton exceeded 150 cc., this tendency has been observed. This serves to confirm the well known fact that at the uppermost layer, i. e. the surface, the multiplication of Diatoms is inhibited due to the excessive intensity of sunlight.

Depth \ Year	Sur-face	2 <sub>m</sub>	4 <sub>m</sub>	6 <sub>m</sub>	8 <sub>m</sub>	10 <sub>m</sub>	15 <sub>m</sub>	20 <sub>m</sub>	25 <sub>m</sub>	30 <sub>m</sub>	Mean
1931	176.2	162.1	178.8	199.1	186.5	191.6	173.4	159.8	137.5	148.9	171.4
1932	293.9	317.0	301.7	305.4	271.3	262.5	263.9	259.3	266.0	261.1	280.7

This table was compiled excluding the two separate collections which were made in Sept. and Oct. 1932.

For the purpose of observing the mean vertical distribution Figure 31 has been drawn from the above data. In both of these two years the general tendency of the plankton to show a higher abundance in the upper layer (down to ten meters) than in the lower ones showed a fair coincidence.

Fig. 31.



Ordinate — volume of plankton (per cub. m.) in cc.

Abscissa — depth in meter.

### 3) Dominating Species

As has been stated the species of Diatoms found in each collection varied from month to month. Again the dominating species of each season varies not only from month to month but from year to year. The species

difference in the corresponding season between 1929 and 1930 was already reported by one of us (KOKUBO, 1932).

Of the three periods of increase in 1931 the first (Apr. 14) was dominated by *Ch. debile* and *Ch. sociale*. *Ch. debile* is one of the most important representatives of Aomori Bay plankton and appears mainly in winter and spring (KOKUBO, 1932). In the second increase (June 15th). *Ch. Schüttii* was the dominating species, *Rhizosolenia hebetata* being second in abundance. The former species is one of the commonest species of *Chaetoceras* in Aomori Bay and predominates at times, in the harvest plankton (KOKUBO, 1932).

Like the vernal increase in 1931 the first increase in 1932 was also represented by *Ch. sociale* and *Ch. debile*. In the second increase of the same year in May *Bacteriastrum* sp. was greatest in abundance as has already been noted by one of us (KOKUBO, 1932) in the following year. The fourth increase which was observed in August was dominated by *Ch. debile* and *Ch. decipiens*. Though the former usually appears in spring and in winter a predominance, in the present case, was shown in summer. The fourth increase occurred in October and was dominated by *Ch. Schüttii* and *Ch. debile*.

As regards the vertical distribution of each species it has already been stated that in some cases species difference can be found between the upper and the lower layers. Besides the data presented here occasional observations often indicated the stratification of *Chaetoceras* and *Coscinodiscus*, the former usually crowding the upper and the latter the lower layer. It also happen, at times, that both the decayed and living cells of *Coscinodiscus Janischii* show distinct stratification. In such a case the empty cell of this Diatom is found in suspension below the layer of living cells.

#### 4) Relation of Plankton to Hydrographical Conditions

Of the seasonal change of plankton the vernal increase of Diatoms has been considered as one of the most significant phenomena as its annual appearance is very regular. This increase of Diatoms usually commences in late January or early February, but a month or so latter it decreases more or less rapidly. As the commencement of the vegetation frequently occurs just after the minimum temperature of water, the vernal increase of Diatoms has been often attributed to the seasonal change of water temperature. This view appears reasonable as the rise of temperature usually accelerates the rate of cell division, but on the other hand BIGELOW

(1924) maintains the view that the vernal increase of Diatoms does not necessarily synchronise with the vernal rise of temperature, based on his observation of the plankton between Cape Ann and the Isles of Shoals where he found the Diatoms commenced to multiply actively while the temperature remained at its winter minimum.

KOKUBO (1932) found in Aomori Bay during 1929-1930 that the vernal increase of Diatoms in 1929 began on February 18th, before the temperature of the water had passed its coldest peak. In 1930 the vernal augmentation commenced on March 7th, following closely the vernal rise of water temperature.

The present research also indicates that in both 1931 and 1932 the vernal increase of Diatoms commenced, earlier than the commencement of the rise of temperature (Fig. 30). In 1931 the first rise of temperature was observed on February 19th, while the vernal vegetation of Diatoms started much earlier than the rise of temperature. That the temperature began to rise around the 20th of February was also demonstrated by the observations made at our pier. The records of the daily observations at this pier, in 1931, show that the lowest temperature, 2.7°C., was recorded on February 20th. In 1932 the vegetation of Diatoms began as early as February 1st, though the rise of sea temperature was first observed on February 15th, showing a delay of about two weeks.

It follows then that in three years out of four the increase of Diatoms preceded the vernal warming of the water, pointing to the conclusion that the rise of the water temperature is not the direct cause of the vegetation of this plant. The temperature change is not likely responsible for harvest increase as autumnal fall of water temperature in three out of 4 years preceded the autumnal fall of water temperature. Regarding the relation between sea temperature and plankton JOHNSTONE and others (1924) state that the change of temperature is a factor that leads to changes in abundance of all planktonic organisms. This statement does not necessarily explain our cases as the vernal and harvest increase, as were indicated above, tend to precede the temperature change of the sea. At any rate the increase or decrease of Diatoms may be the result of the combined effect of temperature, sunlight, salinity, nutrition, and other biological factors. It can not be accounted for by a simple change of sea temperature alone.

## 6) SUMMARY

1) Extending our previous work (1931), observations of the plankton of Aomori Bay was made during 1930 and 1931, by means of the pump

method.

2) It was found that in Aomori Bay in 1931 the Diatoms increased three times during the period from spring to autumn. While in 1932 there were four such increases during the same period.

3) In both these years the first and the last increase seem to correspond respectively to the vernal and harvest vegetation. Summing up the present and the previous (1931) results the harvest increase is by far less prominent than the vernal increase.

4) During the period of the present study the quantity of plankton per cubic meter of water ranged from the minimum of 15.9 cc. (Jan. 19, 1931) to the maximum of 1672 cc. (March 10, 1932), the latter very much exceeding the maximum of 529 cc. of the previous series of observations (1931). The maximum through all seasons and at all depths was 1931 cc. for 6 meter layer observed on March 10, 1932, likewise exceeding the record of the previous investigation.

5) The annual mean of plankto in Aomori Bay was 183.2 cc. in 1931 and 274.1 cc. in 1932 indicating that the annual yield is subjected to changes from year to year.

6) All the data obtained concerning the vertical distribution of Diatoms lead us to the conclusion that when the Diatoms show a normal increase the vertical distribution exhibits the "upper crowding" type. With the decrease of the Diatoms the distribution becomes uniform. When the Diatoms flourish abnormally the vertical distribution becomes irregular, and with the declination of Diatoms the distribution presents the "lower crowding" type.

7) Concerning the relation between sea temperature and Diatomic vegetation the present data indicate that it is improbable that the vernal and the harvest increase of Diatoms is initiated by the seasonal rise or fall of water temperature, suggesting that the change of temperature may not be the direct cause of the vegetation of this plant.

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# ON THE DISTRIBUTION OF GANGLION CELLS IN THE HEART OF THE OYSTER\*

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(With six figures)

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The purpose of the present research is to determine, histologically, whether or not the ganglion cells exist in the heart of the oyster, and if they exist, then to know the form and the distribution of those cells.

Before going further, the writer wishes to express his sincere thanks to Dr. S. HATAI of the Biological Institute of the Tôhoku Imperial University for his kind direction and encouragement, and also to the members of the Institute whose advice has been of great value.

## MATERIAL AND METHOD

*Ostrea circumpicta* PILS. and *O. gigas* THUMB. were used. The former was collected from Mutsu Bay and the latter from Matsushima Bay.

In order to ascertain the innervation of nerves around the heart, the heart and the visceral ganglion with its surrounding tissue were dissected along the dotted line in Fig. 1, and were fixed in various reagents.

For fixation, BOUIN's solution, ZENKER's solution, acetic sublimate, neutral formol, alcohol, ammoniacal alcohol, and potassium bichromate were tried. For staining, DELAFIELD's haematoxylin with eosin, HEIDENHAIN's iron-haematoxylin, MANN's methylene blue eosin, and MALLORY's triple connective tissue staining mixture were applied. As to impregnation, DA FANO's modification of BIELSCHOWSKY, CAJAL method of formula 2 and 3 by LEE ("The microtome's vade-mecum," 1924), and GOLGI method were used.

The heart and the pericardium in several small pieces were embedded either in paraffin or in celloidin and were sectioned 10–20 $\mu$  thick in the former and 50–80 $\mu$  in the latter.

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\* Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 107.



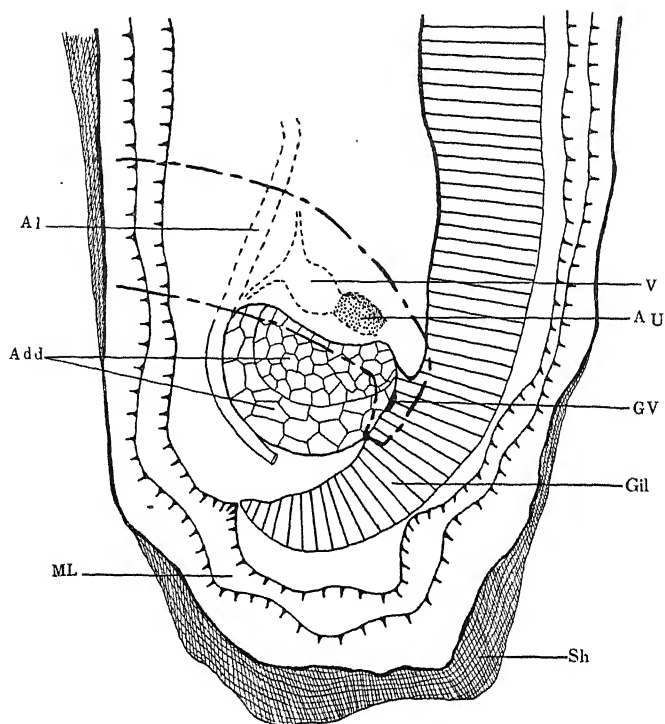


Fig. 1. Diagram to show the relative position of the heart, the visceral ganglion, etc. *Add.*, adductor muscle; *Al.*, alimentary canal; *AU.*, auricle; *Gil.*, gills; *GV.*, visceral ganglion; *ML.*, mantle lobe; *Sh.*, shell; *V.*, ventricle.

At the Asamushi Marine Biological Station, I tried the vital staining with methylene blue "Methylene blue medicinal chem. pure, free from chlorid of zinc" from Merck and Rongalit white. For staining, the tissue was either submerged in a weak solution of the dye or by injecting a more concentrated solution into the body of the living oyster. For stock, a solution of methylene blue 0.5% in distilled water was prepared. 15 to 20 drops of the stock solution were mixed with 100cc. sea water immediately before use.

The heart of the oyster was cut along the median line of the ventral wall, then, on a slice of wet cotton it was spread out under the microscope, the inner side upwards. The tissue thus prepared was left in the dye for 20-26 hours and then was exposed to the action of the air in a moist chamber.

The standard solution of Rongalit white was prepared as follows:

Methylene blue 0.5% in distilled water acidulated in the  
 proportion of 8 drops of 25% HCl to 100 cc. .... 100 cc.  
 Rongalit (obtained from Grubler & Co.) ..... 0.3 gm.

The mixture was warmed in a test-tube until the blue colour changed to pale-yellow, then filtered after cooling. 10 to 20cc. of the stock of "Rongalit white" staining was mixed with 100cc. sea water and the tissue was submerged in this for 3-5 hours.

For injection, I employed the following solution,

Methylene blue 0.5% or Rongalit white (the stand. sol.) .... 1 vol.  
 Sea water ..... 3 to 6 vols.

1-4cc. of this mixture was injected in the heart of the oyster *in situ* and was taken out of the body after 12-24 hours. The heart tissue thus stained was kept in an aqueous solution of 5% ammonium molybdate for a short time and then in a saturated aqueous solution of picric acid. After 1-2 hours the tissue was finally placed in the mixture of picric acid saturated in distilled water and glycerin of the same volume, in which it may be preserved for several months.

#### EXPERIMENT

HEIDENHAIN's iron-haematoxylin gave the most satisfactory results for the innervation of nerves and DELAFIELD's haematoxylin with eosin for the distribution of the ganglion cells.

In *Ostrea*, the heart and the rectum remain apart, the heart lying beneath the rectum. The heart consists of a ventricle and two auricles, the ventricle being twice the size of the auricles. The ventricle is pear-shaped, and when cut open, the wall is seen loosely interlaced by numerous muscle bundles. The auricles are roughly triangular in shape. The walls of the auricles are thin and are only slightly muscular. A glandular epithelium containing a brown pigment invests the auricles which exhibit a dark brown colour.

The blood is a colourless fluid. The blood corpuscles are small and colourless; they are about 10 to 15 $\mu$  in diameter and in permanent preparations show a prominent nucleus in the centre of the rounded or ovoid body.

Advantageously the visceral ganglion is first described, then the innervation of the visceral nerves around the heart and lastly my observations on the nervous ganglion cells in the heart.

The *visceral ganglion*, consists of two parts; fibrous structure in the centre and ganglion cells in the periphery. The fibrous structure is a

mass of nerve fibrils and surround many nerve cells. The nerve cells of the visceral ganglion are unipolar, the bodies of which are approximately round or oval in shape, and measure in average about  $24\mu$  in length and  $10-17\mu$  in width. Thus the nerve cells are far larger than other tissue cells, though those can only be seen by using the high power of the microscope. From one end of a cell body a single nerve fibre arises while the opposite is round. Nucleus of the nerve cell is large and round in shape and measures about  $7\mu$  in diameter, and a clear nucleolus is invariably found in its centre. Protoplasm of the nerve cells stains black with HEIDENHAIN's iron-haematoxylin and dark violet with DELAFIELD's haematoxylin.

Nerves which originate from the visceral ganglion cells exhibit fibrillar structure and apparently lack the sheathing structure. Very rarely, a few nerve cells are found embedded among the nerve fibres, though several cells forming a group are found at the branching place of the nerves.

9 pairs of nerves arise from the visceral ganglion; these are cerebro-visceral connectives, *nervus branchialis*, 2 pairs of *n. adductoris*, *n. pallialis anterior* and *lateralis*, and 3 pairs of *n. pallialis posterior*.

Regarding the distribution of some of these nerves KOGITA (1932) already has given a full account and I shall therefore not repeat it excepting that the oyster heart seems to receive the nerve supply from the first nerve branch of the connectives though I have failed to observe the penetration of this nerve into the heart even when followed through the serial sections.

The anterior pallial nerves are stout cords and, after passing through the nephridia on both sides, runs forward over the wall of the pericardium to the anterior part of the body.

The wall of the ventricle is composed of muscle fibres which are loosely interlaced. The anterior part of the ventricle is divided into two lateral chambers by a septum which consists of muscle fibres. From the posterior end of the ventricle extend two median aorta; anterior and posterior aorta. The walls of the aorta are almost the same in structure as those of the ventricle.

Along the inner-most of the auricular wall are found longitudinal muscle fibres which run parallel to their long axis. The auricles communicate with the ventricle by a narrow slit on each side. The anterior part of the auricles communicate with each other with the pericardial wall.

An examination of the finer structures of the heart wall by various methods resulted in revealing the existence of the ganglion cells (Fig. 2).

These cells are also unipolar and almost the same size as the nerve cells found in the visceral ganglion.

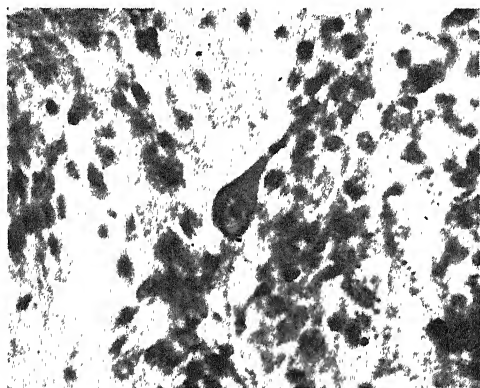


Fig. 2. Photograph of the ganglion cell at the auriculo-ventricular junction.  $\times 700$ .

The nucleus of the ganglion cells is large and round in shape, containing a clear nucleolus in its centre, and the protoplasm takes a dark violet or deep black colour with haematoxylin as those of the nerve cells of the visceral ganglion do.

In the auricles the ganglion cells are observed, mainly, at the region of junction with the ventricle and at the communicating portion with the pericardial wall. At the middle parts the ganglion cells are also found, but very small in number in comparison with the two former parts. The ganglion cells arrange themselves in ring-like formation at the auriculo-ventricular junction.

In the ventricle, there are no ganglion cells in the neighbourhood of the auriculo-ventricular junction, but are mainly at the middle part and at the

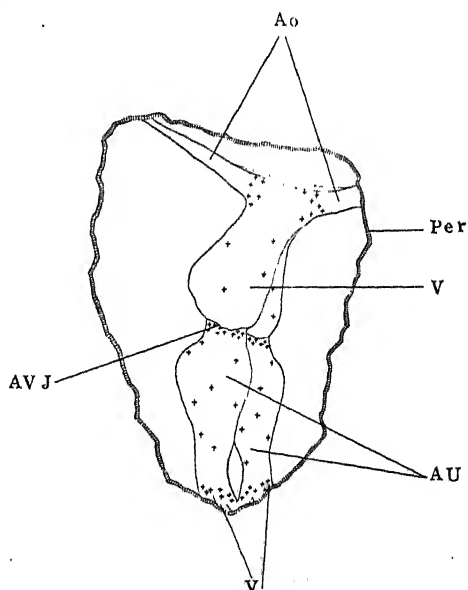


Fig. 3. Diagram to show the distribution of the ganglion cells in the heart.

communicating region with the aorta.

Although the majority of the ganglion cells in the heart is separated from each other by other tissue fibres, some of them were found to be in direct contact.

Figure 3 diagrammatically illustrates the distribution of these ganglion cells in the heart. There are found about 100 ganglion cells in the auricles exclusive of the auriculo-ventricular junction (AVJ) and about 50 cells in the ventricle. At the auriculo-ventricular junction are about 120 cells. A study of the heart wall of the oyster by usual sectioning methods failed to reveal the presence of fibres which can be considered with certainty nerve fibres.

By using the vital staining with methylene blue and with Rongalit white, I was able to observe the nerve fibres in the auricles as shown in Fig. 4. Some parts of the nerve fibres appear to be covered with muscle bundle, but some lie under the muscle and run parallel to those muscles. Not only the fibres are very numerous in number, but make plexus. The nerve fibres after many subdivisions accompany the muscle fibres as very fine fibrils. These fibrils end in the muscle of the septum at the auriculo-ventricular junction without forming any special end-organs (Fig. 5). The ganlion cells lie along the nerve fibres as shown in Fig. 6.



Fig. 4. Photograph to show the nerve fibres in the auricles.  $\times 80$ .

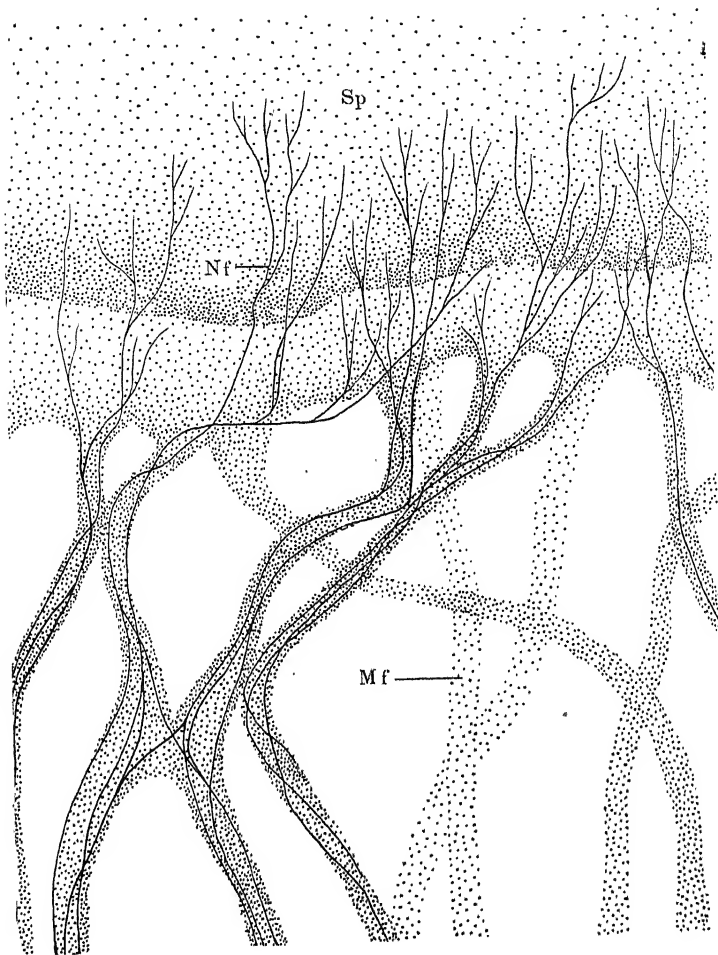


Fig. 5. Endings of the nerve fibres at the septum. (oil immersion).  
*Mf.*, muscle fibre bundle; *Nf.*, nerve fibre; *Sp.*, septum at the auriculo-ventricular junction.

In the ventricle, the presence of the nerve fibres was difficult to determine due probably to the existence of abundant muscle bundles. Whether the nerve fibres in the auricles are the continuation of the nerve which originates from the visceral ganglion can not be determined with certainty, but various circumstances indicate that these nerve fibres is probably in connect with the first branch of the connectives which passes the base of the auricles. I hope that these ambiguous points just mentioned become settled in the near future.

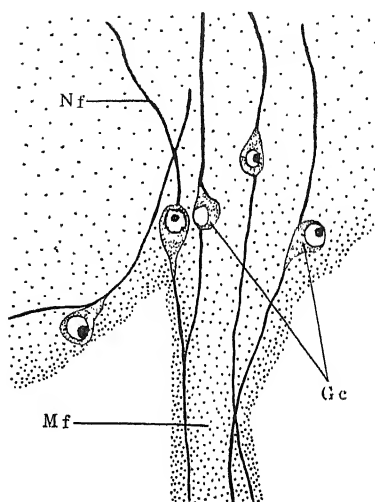


Fig. 6. Ganglion cells and nerve fibres at the auriculo-ventricular junction. (oil immersion).  $\times 750$ . *Gc.*, ganglion cells; *Mf.*, muscle fibre bundle; *Nf.*, nerve fibre.

#### DISCUSSION

KOGITA (1932) found, physiologically, the fact that the heart of the oyster is supplied with the cardio-regulator fibres from the visceral ganglion. According to him, the nerves which enter the ventricle regulate the ventricular rhythm only, and the nerves which enter the auricles regulate the auricular rhythm only. KOGITA concludes from the above that the nerves enter the auricles or ventricle from respective base and the nerves which enter from either bases do not cross the auriculo-ventricular junction.

FUNADA (1926) found with the frog that the heart strip, containing few ganglion cells, give less vigorous response for the same degree of stimulus than the heart strips which contain many ganglion cells which may mean in turn that the muscles in the former still stores a greater amount of potential energy for future contraction than in the muscles in the latter strips after the stimuli were given. From the reasons above mentioned, FUNADA concludes that the heart strips, containing few ganglion cells, are stronger in its resistance than the strips which contain more ganglion cells, when exposed to higher temperature. If this conclusion of FUNADA found with the frog holds true with the oyster heart, then the ventricle of the oyster should show greater resistance to higher temperature than in the auricles, since the latter possesses a much greater

number of nerve cells than the former. But, on the contrary, TAKATSUKI (1932) found that the auricle of the oyster is much stronger in its resistance than in the ventricle when exposed to both lower and higher temperature; that is, in the ventricle, the pulsation is completely inhibited at about  $6.3^{\circ}\text{C}$  and  $38.5^{\circ}\text{C}$  while in the auricle it is inhibited at about  $3.30^{\circ}\text{C}$  and  $43.5^{\circ}\text{C}$ .

The contradicting results obtained by these two investigators might account to the reason that, when the auricle is isolated or cut at the auriculo-ventricular junction, the portion where many ganglion cells are located are necessarily damaged and consequently the number of ganglion cells in the isolated auricle possesses fewer in comparison with the ventricle and hence the auricle, as was shown by TAKATSUKI, is much stronger in its resistance than in the ventricle. To decide this point needs further experimental proof. It should be indicated that the functions of the ganglion cells in the heart strips might not only accelerate, but also inhibit or control the contraction of the heart muscle, and therefore before one accepts FUNADA's conclusion, it seems necessary to make further studies on the various functions of the ganglion cells in the heart strips.

Up to this time, are known many works on the physiology of the isolated heart of the oyster, but according to them, many ganglion cells which are present at both ends are lost, and therefore, the extent to which the lost ganglion cells modified the normal behaviour of the isolated heart, remains to be tested. In studying the lower and higher limiting temperatures on the automaticity of the auricles and ventricle of the oyster, uneven distribution of the ganglion cells in the heart accordingly should be always taken into consideration.

#### SUMMARY

1) The visceral ganglion consists of two parts: central fibrous and peripheral ganglion cell part. The ganglion cell is unipolar and has a large nucleus which contains a clear nucleolus.

2) The nerves which originate from the visceral ganglion and enter the tissue around the heart were observed. The first branch of the cerebro-visceral connectives passes the base of the auricles.

3) The ganglion cells in the heart were discovered and the distribution of these cells were determined. The number of the ganglion cells is larger in the auricle than in the ventricle.

4) Nerve fibres in the auricle were observed by using the vital stain-



ing method. These fibres end in the muscle of the septum at the auriculo-ventricular junction without forming any special end organs.

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# THE NUMBER OF GANGLION CELLS AND NERVE FIBERS IN THE NERVOUS SYSTEM OF THE EARTHWORM, *PHERETIMA COMMUNISSIMA*

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## INTRODUCTION

The present investigation is a continuation of my previous work on the number of ganglion cells and nerve fibers of the earthworm. The results of my earlier studies on this problem are summarized as follows.

1) In the typical segments, the segments without any reproductive organs, the number of ganglion cells in the ventral cord are nearly the same. Similarly the number of efferent fibers in the neuropile as well as in the peripheral nerve trunks are nearly the same in all those segments examined.

2) The number of nerve cells in a ganglion is always less than that of nerve fibers contained in all the nerve trunks arising from the ganglion, amounting approximately to one half of the latter.

3) The presence of the prostate gland is associated with a greater number of ganglion cells and nerve fibers compared with those belonging to the neighbouring segments. In XVIII segment where the prostate gland is located the number of ganglion cells exceeds by far that of the efferent fibers contained in all the peripheral nerve trunks issuing from the ganglion.

4) The number of ganglion cells and nerve fibers in all the segments examined are nearly equal in the right and left halves of the ganglion. When, however, the segment happens to lack any organs in either side, for instance, the worm with non-paired prostate gland, the number of efferent fibers is much increased in the neuropile as well as in the peripheral nerve trunks on the side with the organ, though the number of ganglion cells is approximately the same in both halves of the ganglion.

The conclusion given above was based on the observations made only to the middle parts of the worm, which are caudal to the clitellum

and cephalad to the tail. In order to test the generality of the above conclusion, it is desirable further to determine the following points:

1) The numerical relation of ganglion cells and nerve fibers in the head portion; namely, the cerebral ganglion and the cerebral nerve trunks, the suboesophageal ganglion and all the nerve trunks given off from this ganglion, and the circumoesophageal commissure, which connects the two ganglia just mentioned.

2) The number of the ganglion cells and fibers in the segments anterior to the clitellum, which are complexly organized with various sorts of organs.

3) The number of the ganglion cells and fibers in the tail end segments, which appear to show morphological differences from the typical segment.

#### MATERIALS AND METHODS

The materials used are adult *Pheretima communissima* (GOTO & HATAI), the same species used in my previous work and were collected during the breeding season (Aug. and Sept.). The specimens were at first narcotized by the 1% solution of chlorotone until they became immovable. Then, the viscera were removed by dissection with the greatest caution not to injure the nerves. The nerves were fixed in LAVDOWSKY's fluid together with the body-wall. The materials thus treated were embedded in paraffin, cut transversely or longitudinally 10 or 12 micra in thickness and stained with HEIDENHAIN's haematoxylin. For the enumeration of ganglion cells, I have counted their nucleoli while for the nerve fibers, only the motor fibers were calculated as in my previous works.

#### MORPHOLOGICAL CHARACTERS OF THE WORM

Before the results of the enumeration are mentioned, I will briefly describe some morphological characters of the worm. The part of the body anterior to the clitellum is most complexly organized; there the various organs, digestive, reproductive, circular etc. are specially developed (Fig. 1). The nervous system belonging to this part of the body consists of a bilobed cerebral ganglion, which lies on the dorsal wall of the pharynx, and a pair of circumoesophageal connectives, which pass from the lateral borders of the cerebral ganglion and unite ventrally to the suboesophageal ganglion.

Cerebral nerve trunks: ten pairs of nerve trunks leave the anterior

borders of the cerebral ganglion and innervate into the prostomium and into the wall of the buccal cavity.

The enteric nervous system: five pairs of the small nerve trunks supply the wall of the alimentary tract. Between the ventro-lateral border of the cerebral ganglion and alimentary tract is found one small ganglionic mass in each side namely a pair of ganglionic thickenings of the enteric nerve. The subesophageal ganglion gives rise to eight pairs of large nerve trunks which lie closely to each other. The nerve trunks just mentioned may correspond to single and double nerves of the typical segment.

The ventral nerve cord consists of a row of ganglia united longitudinally by the connective. In each typical segment three pairs of nerve trunks originate from the ventral nerve cord and innervate the body wall. The first (anterior) pair of the three nerve trunks which arises in front of the ganglionic enlargement, is called the single nerve, while the second (middle) and third (posterior) pairs leave the ganglion itself combining with each other into a thick nerve trunk, and are named the double nerve.

In the most caudal segment (tail end) each three pairs of single and double nerves arise from the last ganglion which is greater than the ganglion in the typical segment.

For the distribution of the main peripheral nerves arising from the ganglia in both anterior and posterior segments, the reader is referred to IMAI's paper ('28) who used *Pheretima mega-*

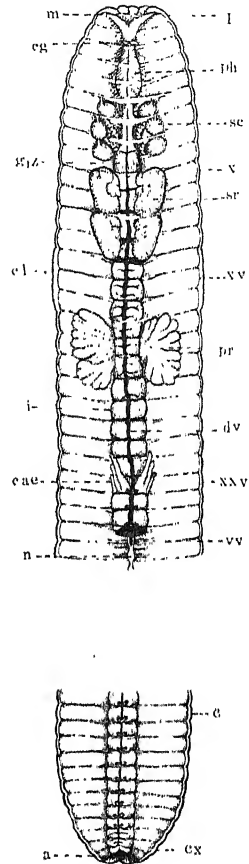


Fig. 1. The internal structure in the anterior and posterior regions of *Pheretima communissima*, showing principal internal organs in a dorsal dissection. I, X, ... CX, No. of segment; a, anus; cae, caecum; cg, cerebral ganglion; cl, clitellum; dv, dorsal vessel; i, intestine; pr, prostate gland; giz, gizzard; m, mouth; n, ventral cord; sc, spermatheca; sr, sperm-reservoir; vv, ventral vessel.

*scolidioides*, which differs little from that of *P. communissima*.

The gross anatomy of the nervous system in the head and tail regions is shown in Figs. 2, and 3.

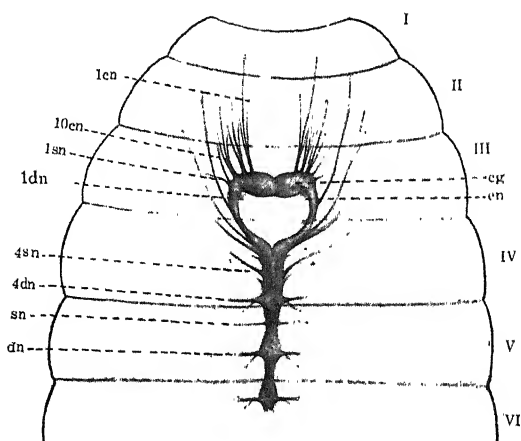


Fig. 2. The nervous system in the anterior segments of *Pheretima communissima*, as seen in a dorsal dissection. I, II,....VI, No. of segment; 1cn,....10cn, 1st,....10th cerebral nerve trunks; 1sn,....4sn, 1st,....4th single nerve trunks of the suboesophageal ganglion; 1dn,....4dn, 1st,....4th double nerve trunks of the suboesophageal ganglion; cg, cerebral ganglion; en, ganglionic thickening of enteric nerve; sn, single nerve trunks; dn, double nerve trunks:  $\times 3$ .

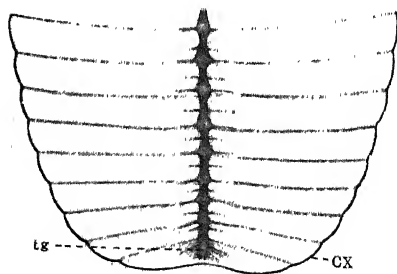


Fig. 3. The nervous system in the posterior (tail) segments of *Pheretima communissima*, as seen in a dorsal dissection. CX, No. of segment; tg, most caudal ganglion:  $\times 3$ .

#### RESULTS OF THE OBSERVATIONS

- 1) *The cerebral ganglion and the circumoesophageal connective.* (Table I)  
The cerebral ganglion has an external cell layer characteristic to the

ganglion constituted by two kinds of nerve cells, one of which appears to be the same as those found in the ventral cord, while the others show special affinity for the staining agents and their cell bodies are not only small in size, but their nuclei are also relatively small.

The neuropile of the cerebral ganglion is densely packed with the endings of longitudinal fibers, which come from the ventral cord by way of circumoesophageal connective. The cerebral ganglion gives off from its anterior border ten pairs of the cerebral nerve trunks to the prostomium as was mentioned already (Fig. 2).

In the nerve trunks are found both the efferent and afferent fibers, which enter this ganglion directly from the periphery; the efferent fibers occupy the greatest area in the cross section of the trunks. In practice, I enumerated all the nerve cells contained in the cerebral ganglion and all the efferent fibers in the circumoesophageal connective and in the cerebral nerve trunks. As Table I shows, the number of nerve cells in the cerebral ganglion is 12829 on the average, namely about ten times as much as the number of nerve cells in the ventral ganglion found in the typical segment, which amounted to 1288.

The total number of efferent fibers in the cerebral nerve trunks is far greater than that in the nerve trunks given off from the ventral cord in the typical segment (single and double nerve trunks combined). Moreover I found that in the larger cerebral nerve trunk the number of fibers is 294, while in the double nerve in typical segment is only 130, as Tables I and II show.

In the circumoesophageal connective I counted 1050 efferent fibers on each side or 2106 in both combined. This number is nearly equal to the number of efferent fibers in the neuropile of the ventral cord.

In Fig. 5, is shown the cross section of circumoesophageal connective, where we notice many efferent fibers together with afferent fibers, as is the case with the neuropile of the ventral cord. No ganglion cells were found in the connective. Furthermore, two pairs of large trunks arise from the middle portion of the circumoesophageal connective which probably correspond in nature to the double and single nerve in the ventral cord, containing 280 and 82 efferent fibers respectively. The enumeration of the fibers in the circumoesophageal connective was performed, at two parts, anterior and posterior where issued two nerve trunks and were found to be 1075 in the anterior, and 1460 in the posterior. From this enumeration we find that the number of nerve fibers in the posterior or before the nerve trunks were issued is larger than in the anterior portion

TABLE  
*The number of ganglion cells and nerve*

No. of segment	The number of ganglion cells	The number of							
		1st nerve		2nd nerve		3rd nerve		4th nerve	
Cerebral ganglion III	12829	Right 207	Left 213	Right 254	Left 251	Right 260	Left 258	Right 203	Left 212
Enteric nerve III	Right 819 Left 824 Sum 1643	37	39	47	48	46	52	50	48
Circumoesophageal connective III, IV		Right 1051		Left 1075		Sum 2126		Single nerve Right 82 Left 85	
Suboesophageal ganglion IV	Right 2582 Left 2564 Sum 5146	Single nerve Right 232 Left 239		Double nerve Ant. 68 Post. 67		Right 207 Left 217		Single nerve Right 48 Left 51	

or after the nerve trunks were issued, giving a difference which is approximately equal to the number of efferent fibers found in the nerve trunks or 362. This result suggests that the most of the fibers in the nerve trunks mentioned above arise from the suboesophageal ganglion.

The ratio between number of ganglion cells and fibers differs in the cerebral ganglion and in the ventral cord. In the former we find the ratio of the number of ganglion cells to that of efferent nerve fibers is 1:2.3, while in the latter it is 1:1.2 even in the largest ganglion (XVIII).

We may be safe to conclude from the above relation that the cerebral ganglion contains a larger amount of associative cells which do not give rise to the large fibers such as those found in the ventral ganglion.

## 2) *The enteric nerve.* (Table I)

As Fig. 2 shows, the enteric nerve thickens in a ganglionic manner at the bottom of the lateral border of the cerebral ganglion, the upper side which is connected by several small nerves, while from the lower side are given off five pairs of small nerves to the pharynx and neighbouring parts of the alimentary tract. The branches which innervate the alimentary tract just mentioned are histologically characteristic, consisting only of fine efferent nerve fibers mixed with a few sensory fibers; that is there are no large nerve fibers such as are found in the single and double

## 1.

*fibers in Pheretima communissima.*

nerve fibers

5th nerve		6th nerve		7th nerve		8th nerve		9th nerve		10th nerve		Sum
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	
288	294	174	169	131	129	58	59	36	34	65	69	3364
		Sum										
48	49	464										
Ant.	t.	Double nerve Post.	t.									
Right	Left	Right	Left									
52	55	280	278									
Ant.	Double nerve Post.	t.		Single nerve		Ant.	Double nerve Post.	t.		Sum		
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left			
71	70	79	82	50	49	94	92	205	203	2124		

nerve trunks. We see, moreover, in the peripheral enteric nerves, as just stated, some large ganglion cells. On the other hand, the number of nerve cells in the ganglionic thickening of the enteric nerve is far less than in the cerebral ganglion, but more numerous than in the ventral cord, namely 819 on the right side, and 824 on the left or 1643 in both combined, contrasted with 12829 in the cerebral ganglion, and 1102 in the ventral ganglion (XXX).

The estimation made by TUGE, for the number of cells found in the enteric thickening was 757 in *Pheretima megascolidioides*, or 92% of that found in *P. communissima* despite the fact that the species used by TUGE is considerably greater in the body size.

We further find in the enteric thickening the ratio of the number of ganglion cells to that of nerve fibers is on the average 464:1643 or 1:3.5, while in the cerebral ganglion it is 5490:12829 or 1:2.3.

In short, the enteric nerves are much smaller than the other nerve trunks not only in the number of nerve fibers but in their diameter. These various differences just mentioned suggest that the physiological properties of the enteric nerve fibers may differ from those of the nerve trunks reaching the muscle of the body wall.

3) *The suboesophageal ganglion.* (Table I)



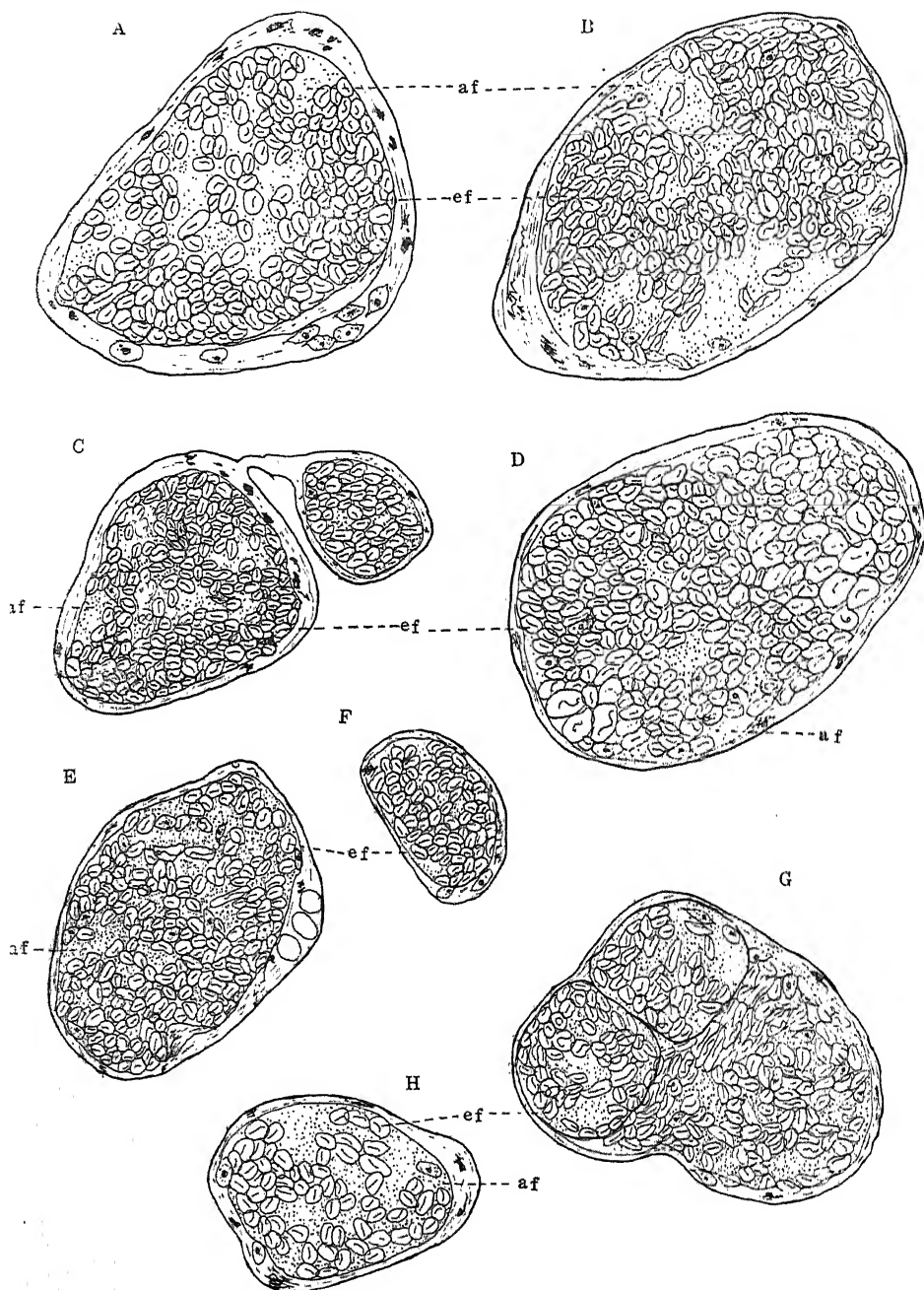


Fig. 4. Cross sections of the cerebral nerve trunks in *Pheretima communissima*, showing the arrangement of nerve fibers,  $\times 600$ . A, B, . . . H, 1st . . . 10th cerebral nerve trunks; af, afferent nerve fiber; ef, efferent nerve fiber.

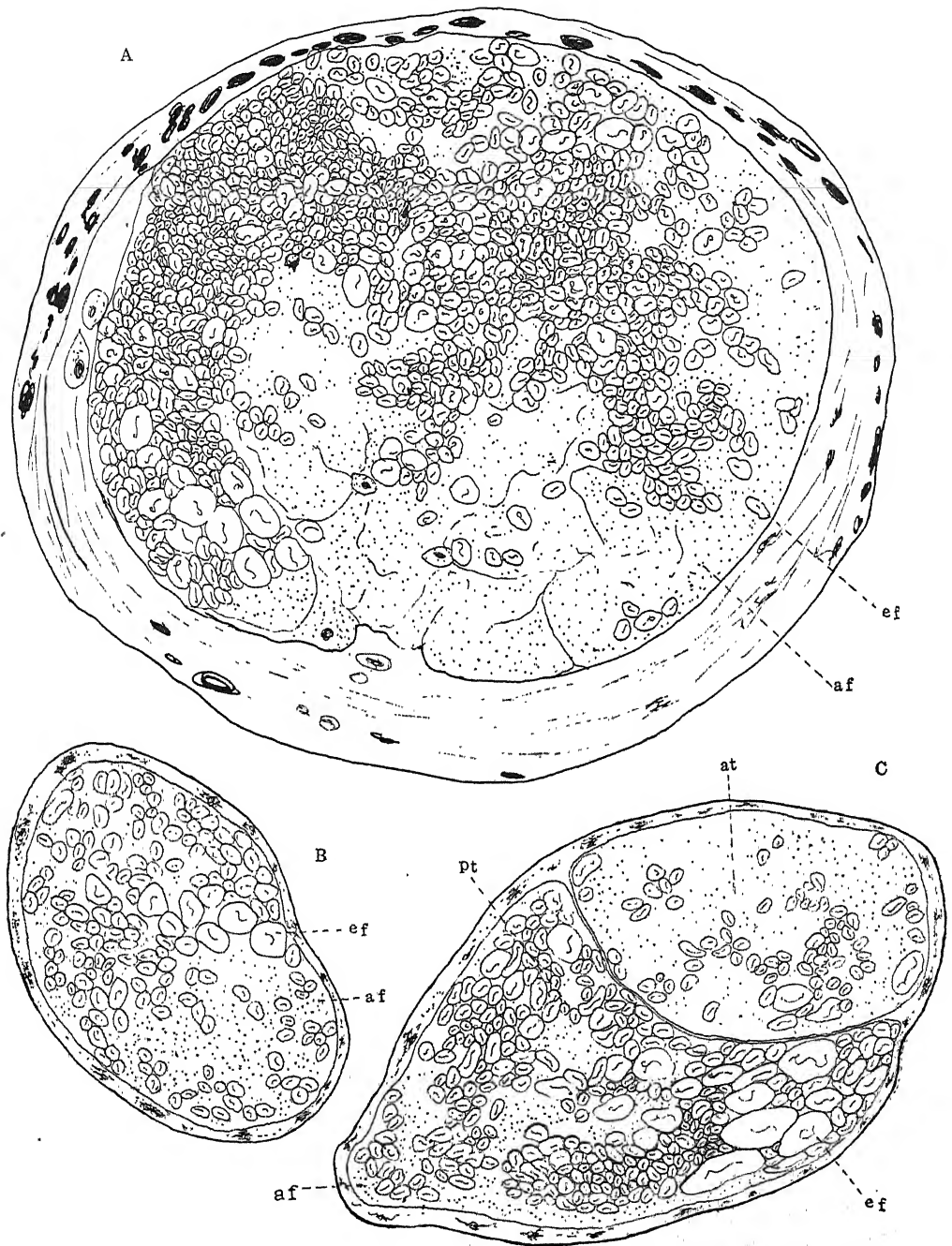


Fig. 5. Cross sections of the circumoesophageal connective, and the single and double nerve trunks given off from the middle portion of it, showing the arrangement of nerve fibers,  $\times 600$ . A, circumoesophageal connective; B, single nerve trunk; C, double nerve trunk; *af*, afferent nerve fiber; *at*, anterior nerve trunk; *ef*, efferent nerve fiber; *pt*, posterior nerve trunk.

The suboesophageal ganglion, which is connected with the cerebral ganglion by a pair of circumoesophageal connectives, is situated in the IVth segment. This ganglion is structurally different from the other ganglia of the ventral cord. It contains many large ganglion cells, though which are arranged in the usual manner. Furthermore, the ganglion cells are uninterruptedly distributed between the suboesophageal and next ganglion of the ventral cord; namely there exists no internode or no demarkation line between them. The giant fibers make their first appearance in the suboesophageal ganglion. Examined embryologically, the median fiber appears in a little earlier stage than the two lateral giant fibers.

From the suboesophageal ganglion four pairs of single and of double nerve trunks arise from each side, as we see in Fig. 2. The 1st pair of single and of double nerves are given off at the middle portion of the circumoesophageal connective, while the 2nd and 3rd pairs leave the ganglion itself, running very closely together; at last, the 4th pair arises at the posterior border of this ganglion.

As Table I indicates, all these nerve trunks are very large, as they have a large number of nerve fibers, the largest trunk giving as many as 275 fibers. The fact that 4 pairs of single and of double nerves arise from this ganglion, means probably that the suboesophageal ganglion is formed by the fusion of 4 ganglia which may be considered in representing the 1st, 2nd, 3rd, and 4th segment.

On the other hand, the number of ganglion cells is in the suboesophageal ganglion far greater than in the other ganglia of the ventral cord, amounting to the sum of 5146; the majority of cells being larger in size.

#### 4) *Anterior ganglia of the ventral cord.* (Table II)

In Fig. 1. the anterior portion (from prostomium to XXIX segment) with various organs is shown. We may naturally expect in the ganglia of these segments the increase of the nervous elements when compared with the typical segments, due to the presence of these additional organs. Really the ganglia before the clitellum have, as Table II shows, a large number of ganglion cells and nerve fibers, though in the IX, X, XI, and XII segments the increase is not so conspicuous as in the more anterior segments. In the clitellum, which is a very specialized portion for the earthworm, a large number of ganglion cells and nerve fibers are found.

According to HARMS, the clitellum may be taken as a secondary female sexual character as its development is completely inhibited when the ovary is removed. I have however found a far greater number of nerve cells and fibers in the clitellar segments than in the proceeding

segments and therefore reached conclusion that this increase might be associated with the greater development of glandular cells in these segments. If HARMS conclusion that the presence of the ovary is responsible for the development of the clitellum holds true, the greater number of nervous elements found by me might rather be connected with still other physiological functions than glandular growth although at present we are not yet aware of any other important function of the clitellum than secretion. Moreover, if the development of the clitellum is controlled both by the ovarian hormone and by the nervous elements as it appears, the removal of the ovary would bring about some reactionary change upon the other element. For the solution of these problems mentioned above further investigations must be done in the future. Among these anterior segments a considerable increase of both the cells and fibers is noted in the XVIII and in its neighbouring ganglia, in association with the presence of a pair of prostate glands.

Contrary to my expectation, the number of ganglion cells and nerve fibers are not increased in the XXVI segment in spite of the fact, that the intestine in this segment has a well developed accessory organ or Caecum (Fig. 1). In remaining segments from the XXI to the anal segment the number of cells and fibers are almost equal in each ganglion,

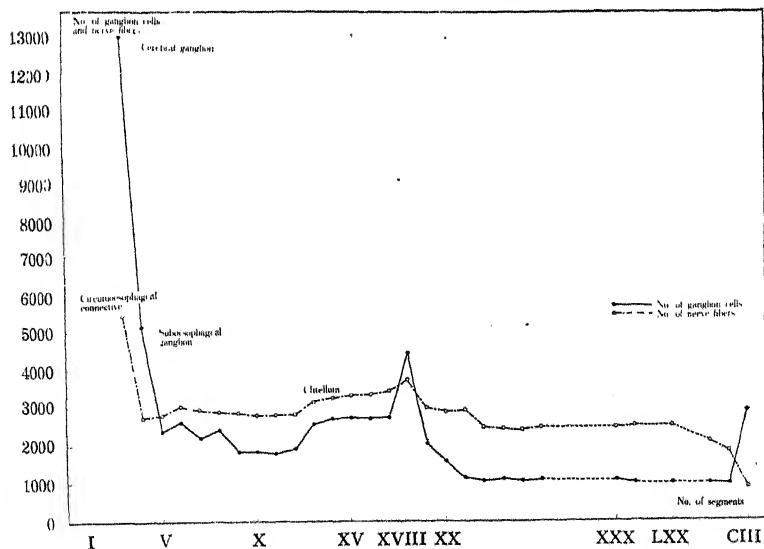


Chart 1. Showing the relation between the number of ganglion cells and efferent nerve fibers in the principal ganglia, extending through from the cerebral ganglion to the most caudal ganglion.

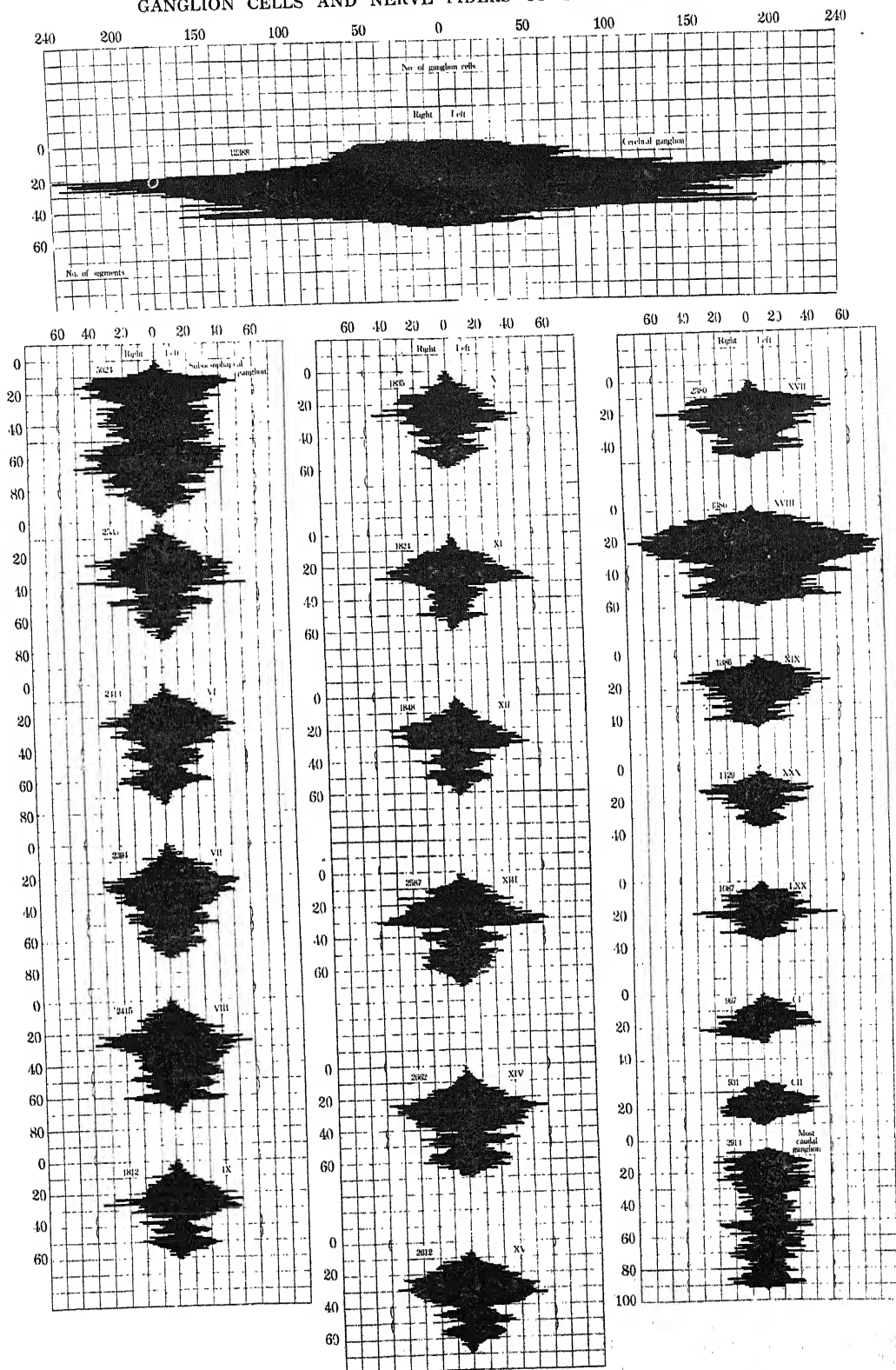
TABLE II.

*The number of ganglion cells and nerve fibers in the middle portion on Pheretima communissima.*

No. of segment	The number of ganglion cells			The number of nerve fibers								Sum
				Neuropile	Single nerve		Double nerve					
							Anterior t.		Posterior t.			
	Right	Left	Sum	Right	Left	Right	Left	Right	Left	Right	Left	
V	1300	1294	2594	1042	1048	284	290	72	139	81	135	3091
VI	1191	1197	2388	1256	1298	180	178	68	125	70	121	3296
VII	1108	1058	2166	1219	1202	75	73	68	127	69	121	2954
VIII	1210	1219	2429	1196	1185	77	73	69	113	70	116	2899
IX	902	917	1819	1193	1187	78	79	71	90	73	86	2857
X	919	908	1827	1180	1170	75	82	65	83	67	84	2806
XI	898	903	1801	1191	1184	74	76	67	79	72	80	2823
XII	961	967	1928	1196	1174	82	73	64	80	60	79	2808
XIII	1329	1336	2665	1318	1315	84	83	87	97	88	98	3170
XIV	1360	1347	2707	1374	1367	91	84	86	98	92	96	3288
XV	1348	1338	2686	1382	1379	92	95	71	122	72	107	3320
XVI	1311	1301	2612	1384	1391	87	89	90	115	92	102	3350
XVII	1321	1322	2643	1418	1409	98	94	94	118	92	122	3445
XVIII	2245	2226	4471	1547	1534	103	106	136	132	133	139	3830
XIX	990	1082	2072	1310	1312	80	83	67	81	65	82	3080
XX	803	793	1596	1251	1243	67	71	63	85	64	84	2928
XXX	569	533	1102	1045	1023	69	72	62	85	64	86	2506

as already described in my previous papers ('28, '30). The numerical relations mentioned above are shown in Charts 1 and 2.

Since it was found by me that not only the number but also the distribution of nerve cells within any given ganglion differs remarkably from others, I have attempted to illustrate diagrammatically those differences just stated in the cerebral, suboesophageal and in several other main ganglia. In Chart 2 the vertical axis indicates the number of the sections cut in thickness of 12 micra, while the horizontal axis indicates the number of nerve cells contained in each section. In addition, where the single and double nerve trunks are given off, is indicated by the brackets. When the number as well as the distribution of nerve cells within each ganglion taken from various portions of body are compared, it will be noticed that those taken from anterior and posterior ends contain generally a smaller



number of the nerve cells. Furthermore in those ganglia just mentioned the sections at the level of the ganglion, where the single and double nerve trunks issue, contains a small number of nerve cells while those sections taken from the anterior half of the ganglion are most crowded with the nerve cells. On the other hand, in the ganglia of typical segments and in those taken from the clitellar segments the distribution of nerve cells in all the sections is similar, and dissimilar distribution such as noticed in the anterior and posterior ends is not found. The most remarkable manner of distribution of nerve cells will be noticed in the cerebral ganglion, suboesophageal ganglion and in the most caudal ganglion or anal ganglion. All these ganglia are formed certainly by the fusion of two or more ganglia. Generally speaking, the number of nerve cells contained, and the mode of cell distribution of the nerve cells in any given ganglion are correlated with the degree of the physiological activity of the segment under consideration, which an appropriate number of nerve fibers must innervate. This in turn demands a corresponding number of nerve cells.

5) *The last, or the most caudal ganglion.* (Table III)

The last caudal ganglion differs from the typical ganglia not only in the mode of dispatching nerve trunks to the periphery but also in the structure of the ganglion itself. This ganglion is kept, so to speak, in

TABLE  
*The number of ganglion cells and nerve fibers in*

No. of segment	The number of ganglion cells.			The number of			
	Right	Left	Sum	Neuropile			
				Right	Left		
XXX	569	533	1102	1045	1023		
LXX	524	520	1044	1019	1043		
CI	497	470	967	898	875		
CII	444	487	931	703	718		
Tail end ganglion	1358	1340	2698	Single nerve		Double t.	
				45 Right	55 Left	49 Right	50 Left

the embryonal condition even in the adult individuals. As Fig. 6 shows, the neuropiles are not clearly outlined and the nerve fibers in them are smaller than those found in the corresponding parts of the typical segments. The ganglion cells, which surround the neuropile, are arranged differently from the other ganglia (Fig. 6, E). On the other hand, the number of nerve cells contained in this ganglion is twice as much as that in a typical segment, as it will be clearly seen in Table III.

Furthermore, we notice that three double nerves and three single nerves, which arise from this ganglion, are kept also in the immature state, inasmuch as the double nerve has not yet clearly differentiated into the anterior and posterior portions, and moreover both the number and size of fibers are far smaller than those in the preceding ganglia (Tables II and III). The single nerves show also an immature condition similar to those shown in the double nerves mentioned above.

Furthermore, the connective between the last ganglion and the preceding one is not clearly marked, so that we can calculate the number of nerve fibers in its neuropile only with much difficulty.

The giant fibers terminate in the middle portion of the last ganglion with the larger lateral fibers and with the smaller middle one.

As indicated in Figs. 3 and 6, the terminal end of the ventral cord bifurcates into two short branches, in both of which fine nerve fibers and

### III.

*the posterior portions on Pheretima communissima.*

nerve fibers.

Single nerve		Double nerve				Sum
		Anterior t.		Posterior t.		
Right	Left	Right	Left	Right	Left	
69	72	62	64	70	66	2471
70	68	51	52	75	77	2455
68	74	56	54	73	71	2169
72	69	48	48	80	82	1820
nerve		Double nerve		Double nerve		Sum
Post. t.	Single nerve	Ant. t.	Post. t.	Single nerve	Ant. t.	
79 82	57 55	44 42	59 61	58 57	45 43	58 52
Right Left	Right Left	Right Left	Right Left	Right Left	Right Left	Right Left



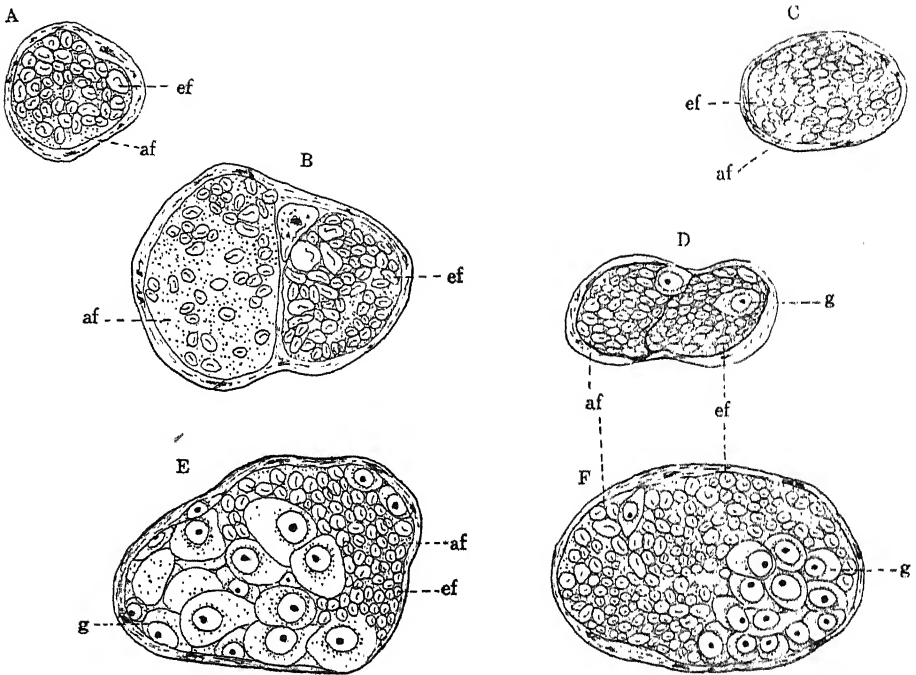


Fig. 6. Cross sections of the terminal branches of the most caudal ganglion, and of the single and double nerve trunk given off from this ganglion,  $\times 600$ . A, 2nd single nerve trunk; B, 2nd double nerve trunk; C, 3rd single nerve trunk; D, 3rd double nerve trunk; E, terminal branch on the right side; F, terminal branch on the left side; *af*, afferent nerve fiber; *ef*, efferent nerve fiber; *g*, ganglion cells.

small ganglion cells are contained.

#### 6) *The size of ganglion cells and nerve fibers.* (Table IV)

While the foregoing observations were carried on, I have noticed that the sizes of ganglion cells and nerve fibers vary remarkably within each ganglion. I shall now present some observations concerning the sizes of the nervous elements. As most of the ganglion cells are pear-shaped, I have measured the two diameters, one along the long axis and other across the widest portion of the cell body for several of the largest and smallest cells in each ganglion. For the nucleus, which is nearly always spherical, one diameter was measured. The difference of sizes are shown in Fig. 7 and Table IV.

Upon examining the figure and the table, we find in the first place that the largest cells in the cerebral ganglion are on the average 28 micra in the long axis, 26 micra in the short one and their nuclei are 10 micra in the diameter, while the smallest cells are 8 micra in the long

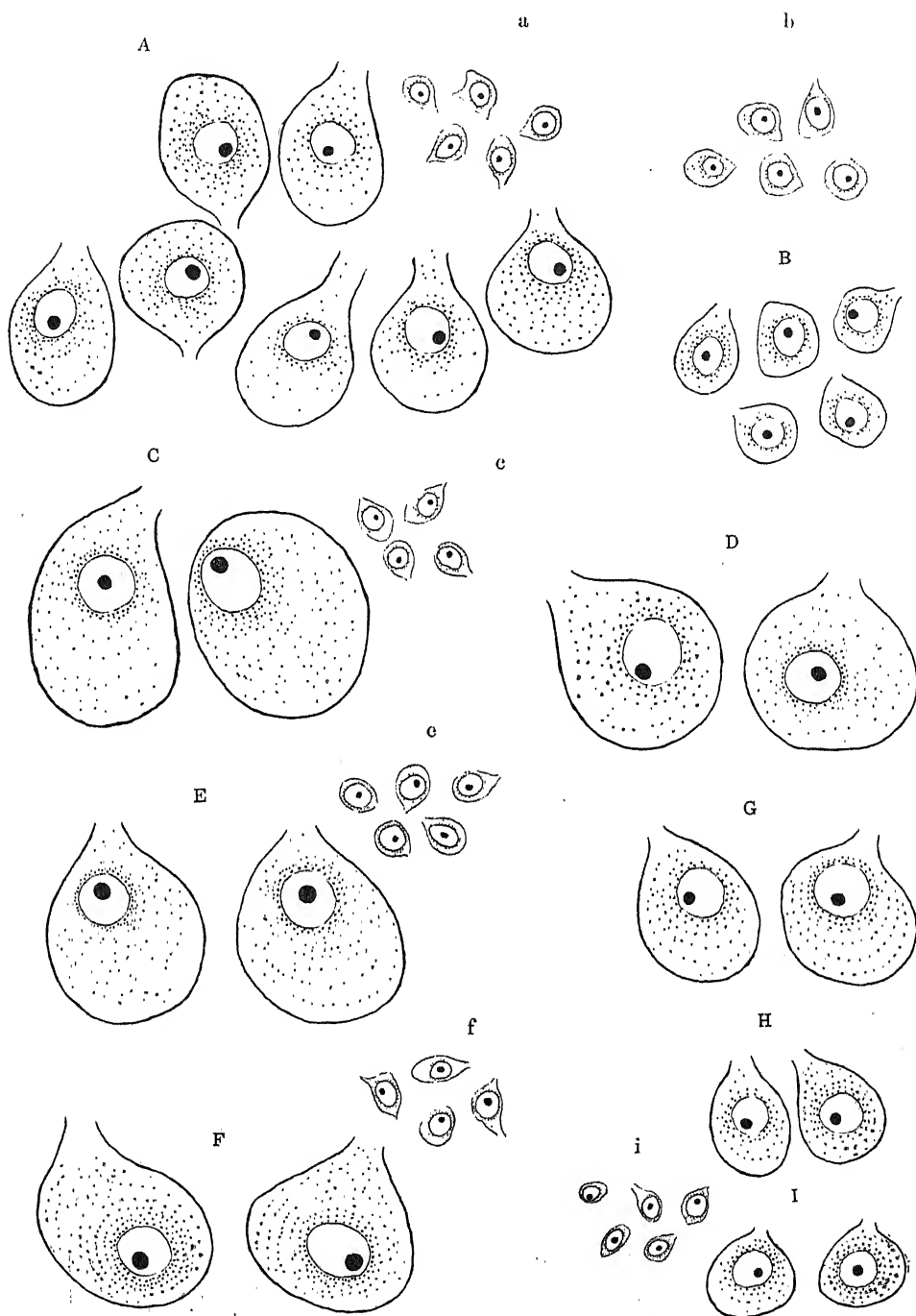


Fig. 7. Comparison of the size of the largest and smallest ganglion cells in several ganglia of *Pheretima communissima*,  $\times 600$ . A, and a, largest and smallest ganglion cells in the cerebral ganglion; B, and b, largest and smallest ganglion cells in the ganglionic thickening of enteric nerve; C, and c, largest and smallest ganglion cells in suboesophageal ganglion; D, largest ganglion cells in the ganglion of Vth segment; E, and e, largest and smallest ganglion cells in the ganglion of XVIIIth segment; F, and f, largest and smallest ganglion cells in the ganglion of XXXth segment; G, largest ganglion cells in the ganglion of Cth segment; H, largest ganglion cells in the ganglion of CIIIrd segment; I, and i, largest and smallest ganglion cells in the last caudal ganglion.

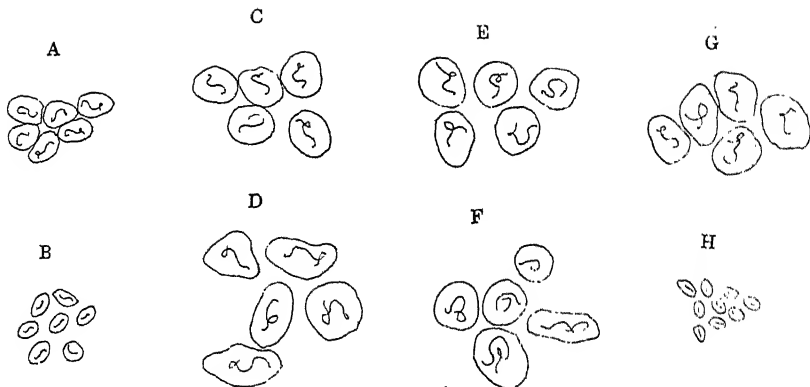


Fig. 8. Comparison of the size between the largest efferent nerve fibers in several nervous regions of *Pheretima communissima*,  $\times 600$ . A, fibers in the cerebral nerve trunks; B, fibers in the enteric nerve trunks; C, fibers in the circumoesophageal connective; D, fibers in the double nerve trunks of the suboesophageal ganglion; E, fibers in the neuropile in Vth segment; F, fibers in the double nerve trunks in XXXth segment; G, fibers in the neuropile of XXXth segment; H, fibers in the most caudal nerve trunks.

and 5 micra in the short axis, and their nuclei are 5 micra on the average.

The suboesophageal ganglion is very characteristic, for it has the largest cells among all the ganglia examined giving as much as  $50 \times 40$  micra in cell body and 15 micra in nucleus. Moreover these large cells are very abundantly present in this ganglion (See Fig. 7 and Table IV). On the other hand, the smallest cells of the suboesophageal ganglion are nearly of the same size as those of the cerebral ganglion. In the ganglia of the ventral cord, we see a pair of largest cells, which are called the giant cells, measuring  $35 \times 40$  micra, the nucleus, 8 micra in diameter. In the most caudal ganglion, which presents an immature form, the small cells are not only much smaller than in other ganglia, but are very abundantly present. The largest cells in the terminal ganglion measure 32 micra on an average thus giving a much smaller value than in the other ganglia.

Regarding the nerve fibers, the largest and smallest fibers measured from various peripheral nerve trunks, the neuropile, and circumoesophageal connective indicate that there are no conspicuous differences in different segments. The cerebral nerves and the enteric nerve trunks are, however, exceptional in this respect; namely, in the cerebral nerve trunks the largest nerve fibers are much smaller than those of the other segments, though

TABLE IV.

*The comparison in the size of ganglion cells and nerve fibers.*

Ganglion	The largest ganglion cells (in micra)	The smallest ganglion cells (in micra)	The largest nerve fibers in diameter	Nerve Trunks
Cerebral ganglion	long axis 25	long axis 9	6 micra	Cerebral nerve trunk
	short axis 21	short axis 7.5		
Enteric nerve	long axis 20	long axis 12	4 micra	Enteric nerve trunk
	short axis 14	short axis 10		
Circumoesophageal connective			9 micra	Circumoesophageal connective
Suboesophageal ganglion	long axis 40	long axis 8.7	12 micra	Double nerve Suboesophageal ganglion
	short axis 32	short axis 7.5		
V ganglion	long axis 34	long axis 8.5	12 micra	Single nerve Suboesophageal ganglion
	short axis 30	short axis 7.5		
XVIII ganglion	long axis 34	long axis 8.6	10 micra	Double nerve XXX
	short axis 30	short axis 7.5		
XXX ganglion	long axis 34	long axis 8.6	10 micra	Single nerve XXX
	short axis 30	short axis 7.5		
Tail end ganglion	long axis 20	long axis 7.2	2.4 micra	Tail end ganglion
	short axis 18	short axis 5		

the smallest fibers are not much different from all others. On the other hand, the enteric nerves consist only of the smallest fibers which are nearly equal in diameter to the smallest fibers found in all other segments. The fact just mentioned gives us additional evidence that the enteric nerves may perform different physiological function from other peripheral and cerebral nerve trunks (See Fig. 8).

In the most caudal ganglion the size of nerve fibers do not exceed 2.4 micra in diameter, we may therefore assume that this ganglion is immature not only as to the cells but also as to the fibers.

7) *General remarks on the number of fibers of the peripheral nerve trunks in the typical segment.* (Table V)

TABLE V.

*The number of nerve fibers in the branches given off from nerve trunks in XXX segment.*

Nerve trunks	Single nerve Right	Double nerve	
		Anterior t. Right	Posterior t. Right
Original trunk	83	79	89
1st branch	24	28	24
Original trunk	59	51	65
2nd branch	16	20	14
Original trunk	43	31	51
3rd branch	13	7	12
Original trunk	30	24	39

In addition I have examined the number of nerve fibers in the single and double nerve trunks, and in the several branches which arise from them. The nerve supply to the body wall was already studied in detail by IMAI ('28), and I have adopted the nomenclature used by him in the present paper concerning the peripheral nerve trunks. Single nerve: This nerve enters, after dispatching three branches to the longitudinal muscle, into the circular muscle layer, but the further tracing of it becomes very difficult. It is usually stated that the single nerve trunks of both sides meet at the ends so as to make a nerve ring, though I could not ascertain the fate of the endings as just stated.

The progressive diminution in the size of the original trunk after issuing the branches is shown in Table V and Fig. 9. As will be seen, the 1st branch of the single nerve trunk is largest among other branches, and the size of the constituent nerve fibers is nearly equal to those in the trunk before branching. The number of nerve fibers in the 1st branch is equivalent to the difference in the number found in the original trunk and the trunk immediately after the 1st branch was given off.

In the same manner, the 2nd and 3rd branches are given off from this trunk, though the sizes of these branches diminish progressively. The numerical difference between the branches and the trunk before and after the branches were given off hold similar numerical relations as mentioned in connection with the 1st branch. Finally the nerve itself enters into

the circular muscle layer, as it was mentioned above.

Concerning the double nerve, we must separately describe the anterior and posterior nerve trunks, because they give off their branches indepen-

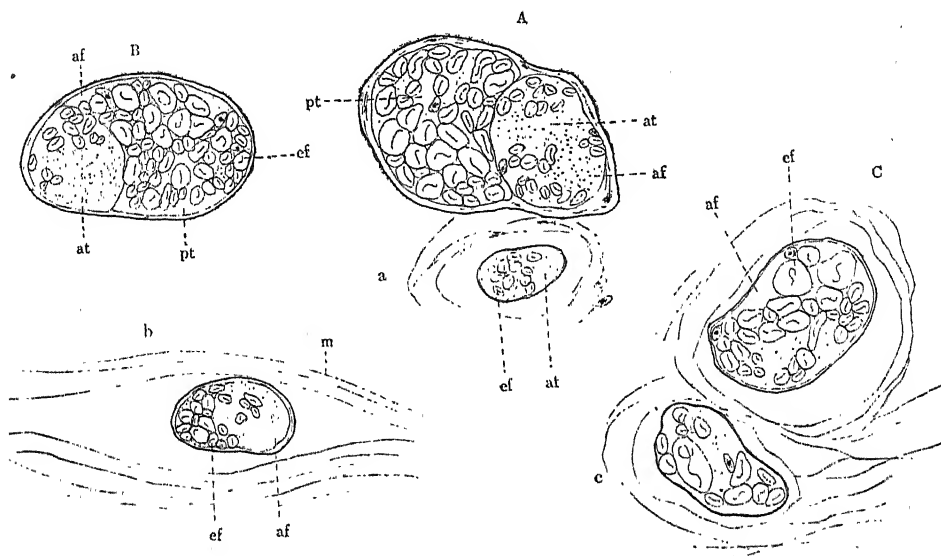


Fig. 9. Cross sections of the single and double nerve trunks (anterior and posterior trunks) and of their branches in XXXth segment,  $\times 600$ . A, double nerve trunk after the 3rd branching of anterior trunk; a, 3rd branch of anterior trunk; B, double nerve trunk after 3rd branching of posterior trunk; b, 3rd branch of posterior trunk of double nerve; C, single nerve trunk after its 3rd branching; c, 3rd branch of single nerve trunk; af, afferent nerve fiber; at, anterior nerve trunk; ef, efferent nerve fiber; pt, posterior nerve trunk.

dently. Soon after they have left the ventral cord, the anterior double nerve trunk gives off the 1st branch which goes to the septum of the segment. This branch consists only of small efferent and afferent fibers, the number of which is 28, as we see in Fig. 9 and Table V. The 2nd branch arises at the same distance from the ventral cord as the 1st branch of the posterior nerve trunk, and enters into the longitudinal muscles. The number of nerve fibers in the 2nd branch is smaller than in the 1st one. Then the 3rd branch is also given to the longitudinal muscle, while the remaining trunk is divided into several branches, which enter the circular muscle layer. The number of nerve fibers in these branches becomes gradually smaller, as we proceed further from the ventral cord. The 1st branch of the posterior trunk is given off at about the level where the 2nd branch is given off by the anterior trunk. Here

are some fibers of the 1st branch accompanying the so called "sensorische Schläuche" which goes to the nephridium. This fact seems to be very interesting, though I can not at present interpret its significance. Several branches arise from the posterior trunk, as from the anterior trunk, but the size of nerve fibers in the branches of the posterior trunk is remarkably larger than in those fibers found in the anterior trunk; even in the distal branch the size of fibers is almost equal to those in the proximal branch (Fig. 9). At last the given nerve trunk reaches the circular muscle layer, where it probably makes the nerve ring.

#### SUMMARY

1) In the cerebral ganglion of *Pheretima communissima* the number of the ganglion cells is far larger than in the ganglion of the ventral cord (Table I); but the size of the ganglion cells is smaller in the cerebral ganglion than in the ventral ganglia. In the cerebral ganglion are seen many characteristic ganglion cells which are not found in the other ganglia.

2) Ten pairs of the cerebral nerve trunks, which are given off from the cerebral ganglion, show some histological differences from the single and double nerves, which are issued from the ganglion of the ventral cord, in such a manner, that in the former the number of the efferent nerve fibers is larger, but the size of them is smaller than in the latter.

3) The number of cells in the cerebral ganglion shows a considerable excess, giving 1 : 2.3 in the ratio of the fibers to the cells. The number of nerve fibers includes the efferent fibers in the circumoesophageal connectives, which may partly take origin in the suboesophageal ganglion.

These results make us assume the existence in the cerebral ganglion of many associative nerve cells, which do not give off their fibers out of the ganglion.

4) The ganglionic thickening of the enteric nervous system consists of relatively smaller ganglion cells.

Five pairs of nerve trunks which arise from this ganglion contain only small nerve fibers.

5) The circumoesophageal connectives are constituted not only by the efferent nerve fibers but by the afferent of two different conducting systems, one of which originates in the cerebral ganglion, while the other takes origin in the suboesophageal ganglion.

6) The suboesophageal ganglion seems to be developed by the fusion of four ganglia, belonging to the 1st, 2nd, 3rd, and 4th segments, as

it gives off 4 pairs of single and of double nerve trunks. The number of ganglion cells in the suboesophageal ganglion is the largest among the ganglia of the ventral cord; also the number of nerve fibers in the nerve trunks dispatched from this ganglion surpasses the number of single and double nerve trunks, issued from any other ganglion of the ventral cord.

7) In the anterior portion (from V to XX segment), which is very complexly organized due to the presence of various sorts of organs, the number of ganglion cells and nerve fibers is much greater than in the ganglia in the typical segments. Generally, the increase of the nervous elements seems to be dependent upon the complexity of the organization in a given segment.

8) The most caudal ganglion appears to remain in the embryonal condition with respect to the structure of the ganglion cells and nerve fibers.

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# SOME NOTES ON THE BEHAVIOR OF THE CATFISH, *PARASILURUS ASOTUS*, AS SEEN THROUGH THE RESPONSES TO WEAK ELECTRIC CURRENT<sup>\*)</sup>

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## PRELIMINARY OBSERVATION

*Parasilurus* is a sluggish, carnivorous and more or less gregarious fish. I made no special observations on the fishes in their natural habitats, but it was noticed that those kept in a small pond assembled together forming a black mass. Whether this assemblage is due to the lack of shelter such as in natural habitats or not is not yet clear. However, it was found that, in a small aquarium in the laboratory a single fish remained there as quiet as those that were assembled. The fishes were placed in a small aquarium in various numbers, but no remarkable differences were noticed but the fishes in a large assembly were more easily to be stirred up, than single or several fishes were kept.

According to PARKER (1917) the American catfish, *Amiurus* is sensitive to metallic rods. When a metallic rod is brought near the fish it responds by either avoiding or turning towards it according to the kind of rods used. It is a well known fact that a metallic rod inserted into water produces a local electric current and the amount of the current thus produced differs by kinds of metals as well as the diameter of rods. With the same metal, the amount of the current produced is proportional to the surface area which is in contact with the water. For the facts just mentioned I used several rods of various diameters made with various metals such as copper, iron and some others. From April to May the reactions of the fishes to those rods were very slight due probably to the cold weather. But from June to July when the water temperature rose to 15°-20°C., the fishes responded to the stimulus as good as in the case of *Amiurus* tested by PARKER. General responses of the fishes to the metallic rods are described below.

Preliminary tests with the rods showed that an iron rod was more

<sup>\*)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 108.

stimulating than that made with copper and furthermore with the same metal, the heavier the rod the stronger is the stimulus. Vigorous avoidance reaction produced upon bringing a heavy iron rod near to them. To a less heavy iron rod of 0.3 CM diameter or so, they responded either by turning away or even approaching towards it, sometimes responding by only moving their barbels. To a thin copper wire brought over the head, no reaction was observed but when shown they turned towards the wire and even nibbling at it. Occasionally some even snapped at the rod as if it were a bait or prey. Thus it soon became clear that the smaller the rod the less is the response and when the diameter of rod is further reduced reactions cease to occur at all. The smallest diameter of the rod to which reactions occur was not determined. It was occasionally observed that some were so sensitive that they even responded to a small piece of fine copper wire (ca. 0.1 mm. in diameter, 10 mm. long) suspended directly over the head by means of a piece of cotton string.

I next tried to stimulate the fishes with a direct electric current from a battery. As an electrode a piece of thin platinum wire mounted on copper wire was inserted into a glass tube of suitable size, its end was projected out for 2 CM. in distance, and both ends of the tube were fused to prevent the penetration of water. These electrodes were kept about 2 CM. apart from each other when brought near to the fish.

The responses of the fishes to electric currents were as sensitive as with metallic rods. To a current of 0.1–0.3 microamperes the fish showed distinct reaction though seldom, only slightly moving their barbels or advancing towards the electrodes, and when the stimulus were withdrawn they soon returned to their former position. These reactions just mentioned are identical with those obtained with a thin copper wire of 0.2 mm. diameter or so. To a current of 5 microamperes or so, reactions occur more frequently, the fishes usually avoid or even approach the electrodes. In many cases they only moved their barbels as though they feel something was nearing them. To currents of 30–50 microamperes the fishes usually ran away from the electrodes, and sometimes very vigorously. But even to those currents reactions often failed to occur. It was not until the current was raised to 300–500 microamperes that the reactions always occurred. At the moment when the electrodes with such a strong current were introduced the fishes ran away with great vigor. But even here the fishes responded sometimes very lazily as if the stimulus were insufficient for them. The similar phenomenon occurs now and then when the fishes were tested with a heavy iron rod.

When several fishes were tested in succession with the same strength of stimulus, some respond more readily than others. The individual variation accounts only partly to the differences shown, since further observations showed that the same fish was sometimes highly responsive, sometimes quieter respond for the same amount of stimulus. Indeed a fish which had responded to a slight current at a certain time fails to respond at all at other times or on the contrary it shows even far greater sensitivity.

Broadly speaking, the grade of reaction is proportional to the magnitude of stimulus and a definite relation between them such as described by PARKER with *Amiurus* seemed to hold good in very special cases with the Japanese catfish.

However to determine these points more precisely and also with a view to accumulate further evidences on the hypothesis recently proposed by HATAI and his collaborators ('32) that the responses of the catfish to earthquakes are brought about by the influence of earth currents, I carried out an investigation in a more definite and systematic way.

#### PRINCIPAL INVESTIGATION

##### *Material and Method*

A wooden aquarium 35 CM long, 25 CM. wide and 20 CM. deep, was partly filled with mud and some waterplants were planted in order to immitate the natural habitats. Five such aquariums were arranged in one row on a wooden sink. In each aquarium two fishes, large and small, were kept for continuous observations. The smaller fishes (11-12 CM. body length) were designated as a-group and the larger ones (16-18 CM.) as b-group. Individual fish was marked either  $a_1, a_2, a_3 \dots b_1, b_2, b_3, \dots$  respectively. Each of the five aquariums contained  $a_1, b_1; a_2, b_2;$  and so on respectively.

The fishes in the aquarium seemed as natural as if they were in their natural habitats, occupying cavities formed in the mud on the darker side, or a space between the roots of plants, etc. Spontaneously or probably by some diturbances they often swim around in the aquarium for a while, usually returning to hiding place previously occupied. The fishes are variable in their times of in habiting the same place, some may occupy the same place for only few days, others for a week or a much longer duration, yet occupation of two or three such hiding places alternately, in general, some seldom stay at any definite place for any length of time.

For instance  $a_1, a_2, b_2,$  possessed no definite place of abode:  $a_1, b_1,$

had two hiding places;  $a_3$ ,  $b_3$ , occupied the same place through out, though once temporarily moving by some disturbances, but returning to the former spot;  $b_4$  occupied the same place throughout the course of the investigation which lasted for a month, sticking himself into a gap made between the mud and glass plates occasionally coming of the shelter but soon returned into it;  $b_5$  formerly had a definite place of abode but afterward roamed around;  $a_5$  belonged to those fishes having a home, but unfortunately died in the early course of the investigation.

From May to June the fishes were often attacked by a fungus, *Saprolegnia*. A fortnight or so after it was placed in the aquarium the majority of the fishes grew weary owing to infection or through some other causes, refusing at the same time any food whatsoever and finally perished. During these seasons it was difficult to keep fishes in a healthy condition. Fortunately in summer when the principal investigation were undertaken, the fishes lived long enough in a sound condition. The fishes were fed with small fish (*Cobitus* sp.) two or three times a week, which they took greedily and maintained good health throughout the entire course of the investigation.

For the purpose of stimulating the fishes, seven rods of pure copper, the diameters of which were 0.5, 1.0, 3.0, 5.0, 8.0, 12.0, mm. respectively (all 15 CM. long), were employed. Each rod was shielded by a glass tube of appropriate diameter and 15 CM. long. The tip of the rod of only 3 CM. was exposed uncovered. The amount of the currents produced by these rods when dipped into the water were estimated roughly, as we have no means for accurate determination in this station, their approximate estimation is; 0.2, 0.5, 1.5, 3, 6, 10, 20, microamperes at 20°C. respectively. These rods were slowly brought about 2 CM. over the head of the fish and held quietly for two or three seconds.

At first the fishes were tested one by one in a definite order with the smallest rod and the responses of the fishes to the rod were recorded. Immediately after the first trial the same procedure was repeated with the rod of next order and the process was continued with the seven rods. When all the seven rods failed to produce a response, further tests were made by an iron rod of 12 CM. diameter. When this rod fails, as it sometimes did, the fish was tested with an iron rod of 15 CM. diameter, to which the fishes so far tested responded without exception.

These procedures were carried out three times a day, in the morning, noon and in the evening, from the 3rd of August to the 1st of September. From the data thus obtained an example is cited in Table 1.

TABLE I.

*Some typical examples chosen from the fundamental data taken every day from the 3rd of August to the 1st of September.*

Body length cm.	Animal	August 6th		
		Morning 23.0°	Noon 23.0°	Evening 23.7°
12	a <sub>1</sub>	1 —	1 —	1 —
		2 —	2 —	2 —
		3 —	3 sl. pos.	3 —
		4 —	4 sl. pos.	4 —
		5 —	5 —	5 —
		6 —	6 sl. neg.	6 neg.
		7 neg.	7 neg.	7 neg.
11	a <sub>2</sub>	1 — 8 neg.	1 —	1 — 8 neg.
		2 —	2 —	2 —
		3 —	3 —	3 —
		4 —	4 —	4 —
		5 —	5 —	5 —
		6 —	6 sl. neg.	6 —
		7 —	7 neg.	7 —
12	a <sub>3</sub>	1 —	1 —	1 —
		2 —	2 —	2 —
		3 neg.	3 —	3 —
		4 barbel	4 —	4 —
		5 —	5 —	5 —
		6 —	6 neg.	6 —
		7 —	7 neg.	7 neg.
12	a <sub>4</sub>	1 —	1 —	1 barbel
		2 —	2 —	2 barbel
		3 neg.	3 —	3 barbel
		4 —	4 —	4 barbel
		5 —	5 —	5 sl. neg.
		6 sl. neg.	6 neg.	6 sl. neg.
		7 neg.	7 neg.	7 neg.
12	a <sub>5</sub>	1 barbel	1 —	1 barbel
		2 —	2 barbel	2 —
		3 barbel	3 barbel	3 —
		4 barbel	4 sl. neg.	4 —
		5 barbel	5 sl. neg.	5 barbel
		6 sl. neg.	6 neg.	6 sl. neg.
		7 sl. neg.	7 —	7 neg.
16	b <sub>1</sub>	1 barbel	1 —	1 —
		2 —	2 — 8 —	2 —
		3 —	3 —	3 —
		4 —	4 — 9 neg.	4 —
		5 —	5 —	5 —
		6 —	6 —	6 barbel
		7 neg.	7 —	7 sl. neg.
		1 pos.	1 barbel	1 —
		2 pos.	2 —	2 —

Body length cm.	Animal	August 6		
		Morning 23.0°	Noon 23.0°	Evening 23.7°
18	b <sub>2</sub>	3 pos.	3 —	3 —
		4 —	4 —	4 —
		5 pos.	5 —	5 barbel
		6 pos.	6 strong neg.	6 barbel
		7 pos.	6 sl. neg.	7 barbel
17	b <sub>3</sub>	1 —	1 —	1 —
		2 —	2 —	2 —
		3 neg.	3 —	3 neg.
		4 barbel	4 barbel	4 —
		5 barbel	5 neg.	5 sl. neg.
		6 neg.	6 neg.	6 barbel
		7 neg.	7 neg.	7 sl. neg.
16	b <sub>4</sub>	1 —	1 —	1 neg.
		2 —	2 neg.	2 neg.
		3 neg.	3 —	3 neg.
		4 neg.	4 neg.	4 neg.
		5 neg.	5 neg.	5 neg.
		6 neg.	6 neg.	6 neg.
		7 neg.	7 neg.	7 neg.
18	b <sub>5</sub>	1 —	1 —	1 —
		2 —	2 —	2 —
		3 —	3 —	3 —
		4 —	4 —	4 —
		5 —	5 —	5 —
		6 —	6 barbel	6 —
		7 barbel	7 barbel	7 neg.

Notes i) 1, 2, 3— are the numbers of the copper rods, diameters of which are 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, 12.0 mm. respectively.

ii) In each trial the fish was stimulated at the interval of one minute or so.

iii) barbel means barbel-moving reaction,

pos. „ positive reaction,

sl. neg. „ slightly negative reaction.

### *Reactions of Parasilurus to the repeated stimuli.*

As will be seen partly from Table I the kinds of reactions of the fishes to the repeated stimuli are so variable that it is difficult to make any simple general statement, but the reactions might be included into any one of the following types.

a) Reactions occur repeatedly several times.

I. The same reaction is repeated.

i) Barbel-moving is repeated.

Examples

1 —	1 barbel	1 —
2 neg.	2 —	2 —
3 barbel	3 barbel	3 —

4 barbel	4 barbel	4 —
5 barbel	5 barbel	5 barbel
6 barbel	6 neg.	6 barbel
7 —	7 neg.	7 barbel
August	August	August
(24th b <sub>4</sub> )	(11th a <sub>5</sub> )	(6th b <sub>2</sub> )

## ii) Positive reaction is repeated.

## Examples

1 —	1 pos.	1 —
2 —	2 pos.	2 —
3 —	3 pos.	3 sl. pos.
4 —	4 —	4 —
5 —	5 pos.	5 —
6 pos.	6 pos.	6 pos.
7 pos.	7 pos.	7 pos.
August	August	August
(7th b <sub>2</sub> )	(5th b <sub>2</sub> )	(5th b <sub>2</sub> )

## iii) Slightly negative reaction is repeated.

1 —	1 neg.	1 sl. pos.
2 —	2 sl. neg.	2 sl. neg.
3 sl. neg.	3 sl. neg.	3 sl. neg.
4 sl. neg.	4 sl. neg.	4 sl. neg.
5 sl. neg.	5 —	5 sl. neg.
6 sl. neg.	6 sl. neg.	6 sl. neg.
7 neg.	7 neg.	7 neg.
August	August	August
(5th a <sub>3</sub> )	(7th a <sub>3</sub> )	(3rd b <sub>3</sub> )

## iv) Negative reaction is repeated.

## Examples

1 neg.	1 —	1 —
2 —	2 —	2 neg.
3 neg.	3 neg.	3 —
4 neg.	4 neg.	4 neg.
5 neg.	5 neg.	5 neg.
6 neg.	6 neg.	6 neg.
7 neg.	7 neg.	7 neg.
August	August	August
(7th b <sub>3</sub> )	(6th b <sub>4</sub> )	(18th b <sub>3</sub> )

## II. Reactions become more and more pronounced.

1 barbel	1 pos.	1 —
2 sl. pos.	2 pos.	2 —
3 sl. pos.	3 barbel	3 sl. pos.
4 —	4 neg.	4 sl. pos.
5 barbel	5 neg.	5 barbel
6 neg.	6 —	6 neg.
7 neg.	7 —	
August	August	August
(16th a <sub>3</sub> )	(14th a <sub>3</sub> )	(21st a <sub>1</sub> )



## III. Reactions become less pronounced finally ceasing.

1 —	1 —	1 —
2 —	2 —	2 —
3 neg.	3 neg.	3 —
4 —	4 sl. neg.	4 —
5 sl. neg.	5 barbel	5 neg.
6 barbel	6 barbel	6 sl. neg.
7 barbel	7	7 sl. barbel
August	August	August
(6th b <sub>3</sub> )	(5th a <sub>4</sub> )	(5th a <sub>3</sub> )

## b) Reactions occur irregularly to the increasing diameters of the rods.

1 pos.	1 barbel	1 neg.
2 —	2 —	2 —
3 barbel	3 barbel	3 —
4 —	4 —	4 neg.
5 —	5 barbel	5 —
6 sl. neg.	6 —	6 neg.
7 —		7 —
August	August	August
(14th a <sub>1</sub> )	(13th a <sub>5</sub> )	(14th a <sub>4</sub> )

## c) Reactions occur more irregularly than in (b).

1 barbel	1 —	1 —
2 —	2 neg.	2 neg.
3 —	3 nibbling	3 —
4 —	4 —	4 —
5 —	5 —	5 —
6 —	6 —	6 —
7 neg.	7 sl. neg.	7 barbel
August	August	August
(6th b <sub>1</sub> )	(12th b <sub>4</sub> )	(15th b <sub>1</sub> )

Besides these eight types given above, there are yet many intermediate forms which cannot be included into any one of the eight types.

The frequencies of each type of response are summed in Table II.

TABLE II.

*The numbers of the types of response to the repeated stimuli.*

Animal	Same reactions occur repeatedly						Irregularly	More irregularly	Intermediate forms	Total cases
	(a)				II	III				
	i	ii	iii	iv						
a <sub>1</sub>	7	2	4	6	13	0	10	9	29	80
a <sub>2</sub>	1	0	1	9	3	2	11	10	43	80
a <sub>3</sub>	0	0	7	31	5	1	11	4	21	80
a <sub>4</sub>	3	0	0	23	4	2	11	5	23	80

Animal	Same reactions occur repeatedly						Irregularly	More irregularly	Intermediate forms	Total cases
	(a)									
	I				II III					
	i	ii	iii	iv						
b <sub>1</sub>	1	2	0	0	3	0	6	11	57	80
b <sub>2</sub>	7	7	0	2	5	0	7	7	45	80
b <sub>3</sub>	0	0	5	55	2	1	7	2	8	80
b <sub>4</sub>	2	1	0	45	1	0	13	8	10	80
b <sub>4</sub>	4	1	3	2	5	2	9	14	40	80
	25	13	20	104	41	8	85	70	276	720

From Table II it is evident that the dominant type of response is that of the repetition of the negative response and that of an intermediate response which comes next in order. It is noticeable that the fish which reacts stronger for increasing size of the rods (II) are relatively less frequent contrary to our expectation.

#### *Individual characteristics*

It is interesting to note that each fish shows a characteristic manner of response, in other words each fish shows individual characteristics towards the stimulus (see Table II). For instance, a<sub>3</sub>, b<sub>3</sub>, b<sub>1</sub> gives Type a iv very frequently but Type b or Type c less frequently. In this connection the behaviors of these fishes in the aquarium are worth noting. These fishes just mentioned stayed at the so-called home showed Type a iv reaction frequently which indicates that they were very responsive to the stimuli. On the other hand a<sub>1</sub>, a<sub>2</sub>, b<sub>2</sub> belonged to those fishes that had no definite place which they occupied for any duration of time and their dominant reactions were the irregular forms of responses of Type b or Type c. a<sub>4</sub>, b<sub>1</sub> had two or three places of abode which they occupied alternately they showed Type b and Type c relatively frequently but lacked types a. b<sub>5</sub> formerly had a definite place of abode for a long time but later roamed about, he showed Type b or Type c frequently which means that this fish was lazy so to speak.

The facts shown above indicate that a fish which readily hides himself by shelter seems to respond regularly to the stimulus. But the reactions found with the fish living in a small aquarium may not always be the same with the fish in the natural habitats.

*Occurrence of various kinds of responses in relation  
to the magnitude of the stimulus.*

The frequencies of different kinds of responses, are summed in Table III.

It was found that the types of the responses of the fishes to increasing diameters of copper rods at first seem highly variable but further analysis

TABLE III.  
*The relation between the occurrence of various kinds of  
responses and the magnitude of stimulus.*

Animal	barbel	pos.	nib.	Slightly neg.	neg.	Strongly neg.	No response	Total trials
a <sub>1</sub>	1	4	5	1	2	—	68	80
	2	7	7	—	3	1	62	80
	3	15	6	—	2	—	57	80
	4	11	5	—	7	3	54	80
	5	21	1	—	3	2	53	80
	6	22	3	—	12	15	25	97
	7	9	2	—	5	38	15	71
	89	29	1	34	59	2	334	548
a <sub>2</sub>	1	—	2	—	3	—	75	80
	2	1	1	2	3	1	71	79
	3	2	1	—	4	—	69	78
	4	4	—	—	7	—	68	79
	5	3	1	—	9	—	62	78
	6	11	—	—	2	1	38	71
	7	5	—	—	1	35	29	70
	26	5	2	8	80	2	412	535
a <sub>3</sub>	1	—	—	—	13	—	64	78
	2	1	—	—	5	—	59	74
	3	2	—	—	6	—	38	71
	4	1	—	—	7	—	40	75
	5	—	—	—	6	—	39	75
	6	1	—	—	9	1	16	72
	7	—	—	—	8	—	8	62
	5	0	0	42	204	1	255	507
a <sub>4</sub>	1	2	1	—	1	4	72	80
	2	4	—	—	1	5	68	78
	3	5	—	—	1	10	64	80
	4	4	—	—	2	18	50	74
	5	7	—	—	5	20	39	71
	6	6	1	—	6	37	22	72
	7	8	—	—	3	45	9	66
	36	2	0	10	139	1	324	521

Animal	barbel	pos.	nib.	Slightly neg.	neg.	Strongly neg.	No response	Total trials
b <sub>1</sub>	3	1	—	—	3	1	12	80
	2	1	—	2	2	—	73	80
	—	1	—	1	1	—	77	80
	2	5	—	1	—	—	72	80
	4	1	—	3	—	—	67	75
	9	3	—	5	3	—	55	75
	7	5	—	6	10	1	38	67
	27	17	0	18	19	2	454	537
b <sub>2</sub>	5	1	—	—	—	—	74	80
	3	1	—	—	—	—	73	77
	2	4	1	—	—	—	73	80
	2	—	—	1	2	—	70	75
	7	2	—	—	2	—	60	71
	11	7	—	2	2	—	45	67
	16	12	—	3	9	—	38	78
	41	27	1	6	15	0	433	528
b <sub>3</sub>	—	2	—	1	36	—	41	80
	3	—	—	4	34	2	34	77
	4	1	—	7	42	1	25	80
	5	—	—	7	48	—	20	80
	2	—	—	8	54	—	8	72
	3	—	—	9	63	1	3	79
	—	—	—	5	74	1	—	80
	17	3	0	41	351	5	132	549
b <sub>4</sub>	2	—	—	3	26	3	47	81
	3	—	—	4	32	—	40	79
	3	—	—	5	40	1	30	80
	3	—	—	3	44	—	24	74
	3	—	—	4	46	—	23	76
	4	—	—	2	50	—	14	70
	—	—	—	3	56	3	10	72
	18	0	0	24	294	7	183	532

Notes I) 1, 2, 3 — are the numbers of the copper rods, of which the diameters are 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, 12.0 mm. respectively.

II) barbel means barbel-moving.

pos. „ positive reaction.

niv. „ nibbling.

show that the responses are more definitely related with the magnitude or the stimulus (Table III), that is, the cases of the negative responses increase as the stimulus becomes stronger, conversely the positive responses become more frequent with decreasing stimulus. Barbel-moving reactions seemed to occur almost independently to the magnitude of the stimulus, within the limit of stimuli applied. As to the nibbling reactions no

definite relation could be found as it occurred seldom.

Strongly negative responses should occur naturally to stonger stimuli, but as are shown in the table the reactions occurred not only to the weaker stimuli but even to the weakest. Often the reaction fails to follow with the rod of the next size after the first reaction occurred with the rod of the smallest size, but reoccurs only when much stronger stimuli were applied. This means probably that when the fishes are stimulated at short intervals, they become accustomed or fatigued and do not respond to moderately increased stimuli, but as the stimulus become much stronger they become vigorously responsive again.

What is described above indicates that the reactions vary with the magnitude of the stimulus. As to whether or not these variable results were due to the mode of testing or repeated stimuli at short intervals, can not be settled definitely, until the fishes are tested at much longer intervals. During the course of experimentation we met with another complication due to the habit formation to the stimulation. For I encountered occasionally with some fish which when tested often with stronger stimuli, apparently formed a habit of exhibiting negative response not only to the metal rods but even to the baits brought near them. Some other fish which showed a negative response to the given size of metal rod approach towards the same rod if kept undisturbed for a long time, but was occasionaly fed with earthworms or with live fishes.

The present work was undertaken at the suggestion of Prof. S. HATAI, whom I wish to express my hearty thanks for his kind guidance in the course of the work. I wish also to thank the Saito Hoon Kai (Saito Gratitude Foundation) for financial aid.

#### SUMMARY

1. The responses of the catfish, *Parasilurus asotus*, to the repeated stimuli with the copper rods, show considerable variation. But the responses may be classified into several types.
2. The kinds of responses are dependent generally on the magnitude of the stimulus given.

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# ECOLOGICAL OBSERVATIONS ON *BATILLARIA* *MULTIFORMIS* (LISCHKE) \*

By

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(with 7 figures)

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## INTRODUCTION

*Batillaria multiformis* (LISCHKE) is commonly seen on sand shores and on rocks covered with sea weeds (*Gloiopelis furcata*, var. *intricata*, SWR.) near the Mitsui Institute of Marine Biology at Susaki on the Izu Peninsula. This gastropod belongs to Platypoda of Prosobranchia, and are found forming groups on rocks though it does not colonize like *Acmaea dorsuosa* GOULD (ABE, 1933). Their habitats are varied being found in the sea, on the exposed rock, in the sand and also in the tide-pool. So I thought that it will be very interesting to compare its behavior with *Acmaea*. In this paper I have described, mainly, the movement of this mollusc.

This work was carried out at the Mitsui Institute of Marine Biology since the middle of May to the beginning of July in 1933. Here I wish to express my sincere thanks to Baron K. MITSUI for affording me this opportunity for research work and for his good wills in many ways. And I wish to express my hearty thanks to Prof. Dr. S. HATAI for his kind direction and suggestions given during the work.

## HABITAT

*Batillaria multiformis* inhabits the region between high and low tide mark, or the littoral zone. As to the number of individuals found in any given locality, it was found to differ according to the habitat; rocky shore, pebbly shore and sandy shore. The following figures were obtained in one square metre at the regions where the shells were most crowded.

Rocky shore — 529; Pebbly shore — 115; Sandy shore — 64.

On the rocky shore habitat, the snail is seen only where the rock is

\* Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 109.



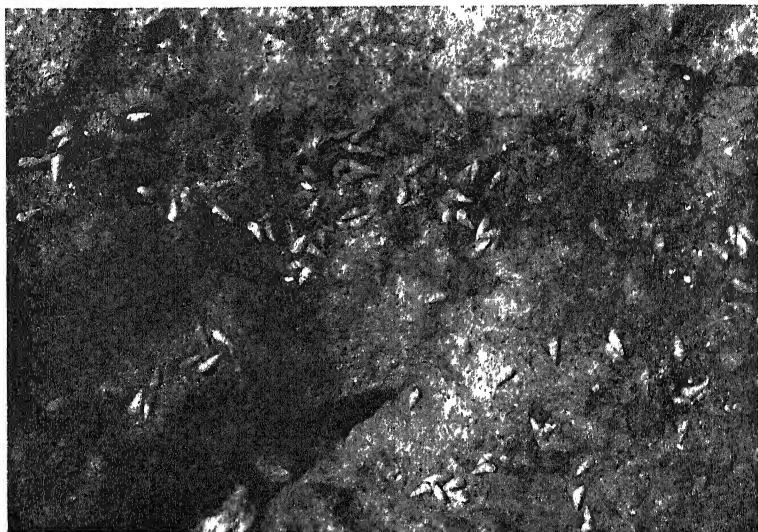


Fig. 1. *Batillaria multiformis* in the rock habitat.  
(photographed on June 4, 1933, 9:40 am.)

covered with *Gloiopelis furcata*, var. *intricata* in summer (Fig. 1), but in one occasion after a stormy night they were found on rocks even where sea weeds do not grow. This snail is seen both on the light and on the dark side of the rock and thus differs from the behavior of *Acmaea* (ABE, 1931) and of *Monodonta* both which live on the darker side normally. The direction of the shell on the rock is about constant showing head side up and apex of the shell down. The positions on the sandy shore are so complicated that it will be described in the later pages of this paper.

#### METHOD OF LOCOMOTION

Unlike that of *Littorivaga* and *Acmaea* the propagation of waves on the foot surface of this species can not clearly be seen owing to the presence of dark brown pigment. However the method of differentiation of locomotion by VLÈS (1907), *Batillaria* belongs to monotaxic direct movement. Characteristic locomotion of most snails is smooth movement by slow gliding only as will be seen with *Monodonta*, *Littorivaga* and *Nerita*, but *Batillaria* moves by slow gliding mixed with leaping in regular intervals as in *Strombus* (PARKER, 1922). A kimographic record of the leaping is shown in Fig. 2.

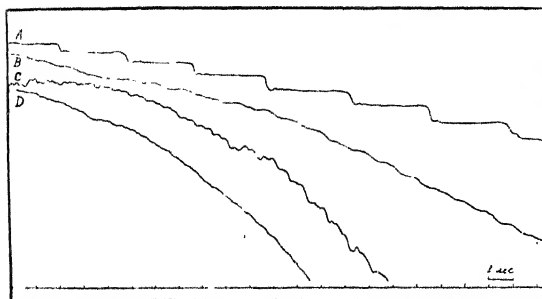


Fig. 2. Kimographic record of creeping of *Batillaria* and other animals.<sup>1)</sup>

- A. *Batillaria multiformis* (LISCHKE).-Uminina.
- B. *Nerita* (*Heminerita*) *japonica* DUNKER.-Amagai.
- C. *Japeuthria ferrea* (REEVE).-Isonina.
- D. *Monodonta* (*Melagraphia*) *neritoides* (PHILLIPPI).-Kurozukegai.

On the number of leapings, 11 to 15 leapings per minute in the field and 11 to 33 leapings per minute in the laboratory at about 28°C. were noted.

## BEHAVIOUR

Here, the behaviour of *Batillaria* in the sandy shore habitat will be described. For this purpose I have made a frame of one square metre with partitions in every 20 centimetres and determined the number of individuals in each partition together with the direction of creeping and other properties. Each observation involves the records taken from five different places on the sand shore and then averaged.

### 1. Time of creeping

I wished to find out the time when *Batillaria* creeps for feeding or for other purposes. I have observed every day at the sand shore and it became clear that it creeps only in the time of low-tide as shown in Fig. 3. In Fig. 3, it is seen that *Batillaria* creeps as the sand begins to become exposed or two or three hours before the lowest tide was reached, the manner of creeping will be described in detail.

At the moment the sand begins to appears with the ebb-tide, *Batillaria* begins to raise the sand a little and 20 or 30 seconds after it again lifts

<sup>1)</sup> On the identification of these snails I am greatly indebted to Mr. T. KURODA. Here I wish to express my sincere thanks to him.

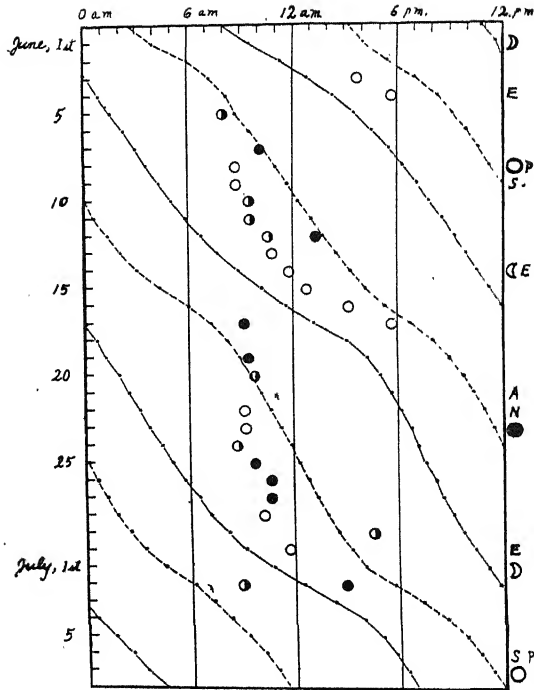


Fig. 3. Locomotion of *Batillaria* in relation to tide.

- high-tide.      ..... low-tide.  
 ○ individuals are all in creeping.  
 ● individuals buried their bodies in the sand.  
 ◐ half of all the numbers are in creeping and the rest are buried in the sand.

up the sand, followed by a spiral movement of body as though the shell is screwed out of the buried sand. Finally the entire body appears on the sand and before it begins creeping the shells are usually rolled several times by the waves. The following will illustrate the statement just given.

June 11th,

- 9:41 am....*Batillaria* lifted the sand.  
 :42 50 ....Entire body appeared.  
 :43 30 ....Lies horizontally on the sand.  
 :43 45 ....Rolled by waves.  
 :44 16 ....Resumes normal position and begins creeping.

*Batillaria* feeds on diatoms and algae on the sand while creeping. But 20 or 30 minutes after staying on the sand it begins to prepare for burying its body again in the sand in boring a hole by the end of the operculum, and creeps in from the head side, as is shown in Fig. 4.

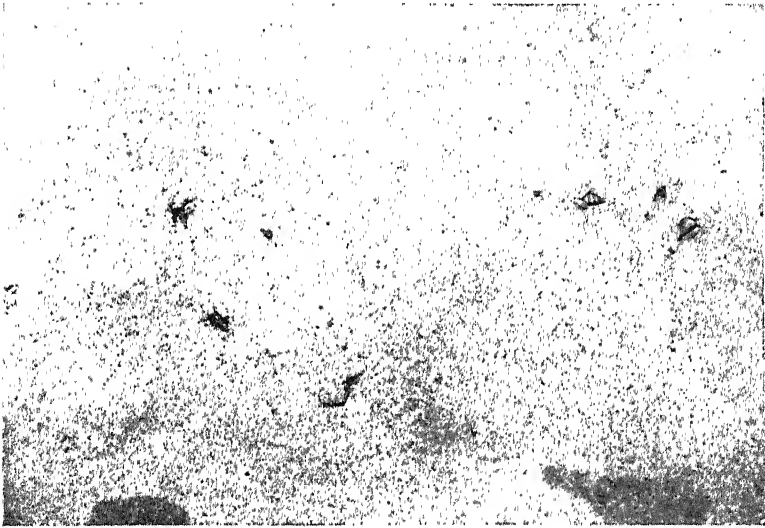


Fig. 4. *Batillaria multiformis* crept in the sand.  
(photographed on June 4, 1933, 9:50 am.)

*Batillaria* thus burried in the sand do not move at all until the tide flows and the entrance-marks made by the snails are obliterated by the waves. At the time of high-tide, water over the sand habitat reaches a depth of 50 or 60 centimetres. I do not know whether during the time of high-tide it creeps under the sand, as I have never met a single instance of creeping on the sand.

*Batillaria* are found under the sand, at a depth which is less than three centimetres and the majority of them were 1 to 1.5 centimetres in depth. I have seen individuals lie quietly, cover their bodies lightly with sand and especially the head end very thinly. A little hole is invariably found near the head of each such burried individual (Fig. 5). On the 17th of May, I have noticed 33 individuals each having one hole and only one individual having no hole at all. This hole probably serves the purpose of respiration, but this requires further investigation.

On days when the sun shines strongly and the sand dries quickly, *Batillaria* burries only its head end into the sand.

It is noticeable that when low-tide occurs twice a day, once in early morning and once in the evening, as on the 17th of July, only 4 individuals per one square metre were found creeping in the morning, while 42 individuals per one square metre in the evening. On the 18th, creeping individuals were more numerous in the evening than in the morning. At

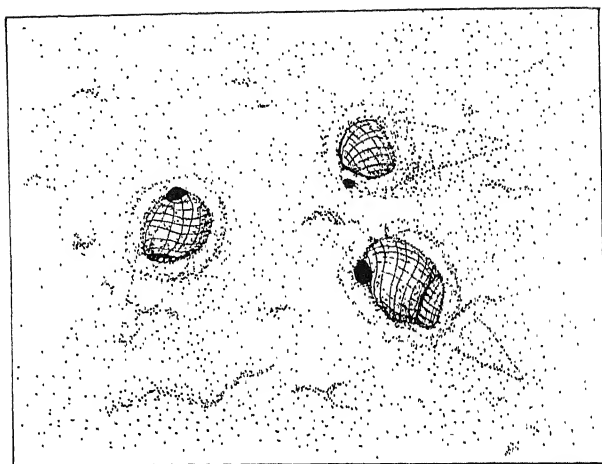


Fig. 5. *Batillaria multififormis* buried in the sand with a small hole.

night or on a brightly shining moon-night, the species do not creep at all even at low-tide.

## 2. Direction of creeping

I saw many *Batillaria* creeping landward from sea on the 25th of May, on the 3rd of June, I saw their creeping seaward from land. So I wished to know what induces them to creep either landward or seaward. I have thus observed since the 3rd of June, the direction of creeping every day for nearly one month. The results are shown in Table I.

TABLE I.  
*Direction of creeping of Batillaria*

Date	Time	Atmos- pheric tempera- ture in C°	No. of inds. per one metre square	No. of inds. creep in the sand	A Direction of creeping in degrees	B Direction of sun light in degrees	A~B in degrees
June 3	3:50 pm		44.4	1.0	155	150	5
„ 4	5:40 pm		26.0	0	176	174	2
„ 5	8:00 am		16.0	3.6	88	30	58
„ 6	—		—	—	—	—	—
„ 7	10:10 am		0	0	—	—	—
„ 8	8:50 am		9.0	0.4	65	41	24
„ 9	8:50 am		15.6	0.8	55	41	14
„ 10	9:30 am	23.0	16.0	3.4	57	51	6

Date	Time	Atmos- pheric tempera- ture in C°	No. of inds. per one metre square	No. of inds. creep in the sand	A Direction of creeping in degrees	B Direction of sun light in degrees	A~B in degrees
June 11	9:30 am		15.8	1.4	74	51	23
„ 12	10:30 am		27.6	2.2	82	68	14
„ 13	10:50 am		25.6	0	101	79	22
„ 14	11:50 am		35.4	0	79	86	7
„ 15	0:50 am		22.6	0	148	103	45
„ 16	3:10 pm		25.2	0	137	137	0
„ 17	9:15 am		3.2	1.6	—	53	—
„ 17	5:40 pm		41.4	0	—	175	—
„ 18	—		—	—	—	—	—
„ 19	9:25 am		14.2	7.4	—	49	—
„ 20	8:40 am	26.0	10.4	5.6	53	38	15
„ 21	—		—	—	—	—	—
„ 22	8:10 am		4.2	0.2	49	32	17
„ 23	9:10 am	23.0	2.0	0	—	47	—
„ 24	8:40 am		17.8	6.8	44	40	4
„ 25	9:50 am	26.0	6.4	5.4	—	46	—
„ 26	10:40 am		7.8	6.8	—	69	—
„ 27	10:40 am	27.0	7.6	5.4	75	70	5
„ 28	10 10 pm	25.0	10.0	0	49	62	13
„ 29	2:40 pm	24.0	12.2	3.0	—	130	—
„ 30	11:50 am	28.0	12.4	2.0	83	86	3
July 1	—		—	—	—	—	—
„ 2	9:00 am	29.0	11.4	6.8	134	134	0

In Table I, the number of individuals which were either creeping or those which were creeping out or into the sand were recorded. The direction of creeping was recorded with respect to the position of the sun. As to the direction of sun-light, 0° signifies that it shines from east to west, from south to north by 90°, and from west to east by 180°. From Table I, we see that the direction of creeping accords with the movement of the sun light within the deviation of about less than 20°. For this reason it is clear that the direction of creeping in the morning is quite opposite to that in the evening.

### 3. Number of individuals in creeping in relation to moon (Migration).

In Table I, it is clear that the number of *Batillaria* which were creeping on the sand changes every day. I have also recorded the number

of newly migrated molluscs on a small rock for the purpose of determining the factors which may modify the number of migrators. The size of the rock chosen for this purpose is 1.3 metres in length, 0.6 metres in breadth and 0.2 metres in height, and is separated by sand from the beach cliff by only 70 centimetres. The results of the observations are shown in Fig. 6 together with the change of number of individuals creeping on the sand.

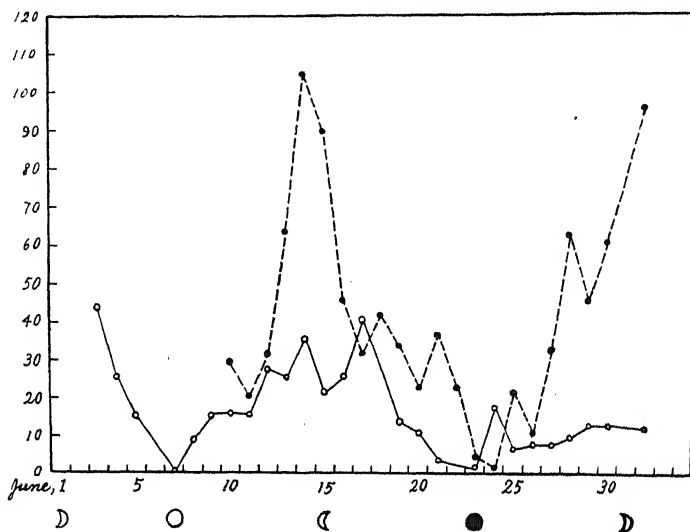


Fig. 6. Number of individuals in creeping in relation to moon (migration).

●---● individuals migrated to a rock.

○—○ individuals crept on the sand.

○ full moon.

● new moon.

( or ) half moon.

In Fig. 6, we see that number of individuals creeping on the sand vary considerably and the minimum numbers were found on the 7th and the 22nd of June. The 7th is a day of full-moon and the 23rd is a day of new moon. While on the days of half-moon, the number of individuals was most numerous. On the 7th and 21st a very heavy rain fell and the rest of days were fair. It seems at first, that the fewer number of individuals found creeping on the 7th and 21st may be attributed to an influence of rain fall as for instance J. D. HASEMAN<sup>1)</sup> (1911) found with the movement of *Litorina litorea*, but on the contrary the reduction of

<sup>1)</sup> J. D. HASEMAN (1911) found with the movement of *Litorina litorea*, that "When it rains on exposed snails, they become quiescent just as they do when splashed by the water. Therefore during stormy as compared with fair days, a considerable difference in the amount of oscillations of *Litorina litorea* was noted."

numbers with our case was already seen about two days before the rain fall. So I think that the periodical increase or decrease in the number of individuals is more likely related to the moon or to the tide.

#### 4. *Movement of Batillaria affected by light*

*Batillaria* creeps in a direction opposite to sun light as stated above, and this reaction was found in about 95%. Therefore it is clearly evident that *Batillaria* shows a nature of negative phototropism in the field. I now wished to determine how they would adjust themselves (when they were placed in either dark or in poorly lighted rooms) and the following experiments were carried out.

In the middle part of a dark room some 5 metres square, 4 glass plates of 30 centimetres square were laid horizontally, and one *Batillaria* is placed on the centre of each glass plate and one or half hours later their traces were examined. This trace is clearly printed by a kind of mucus secreted by the snail. When light is needed, the curtain which hangs in front of a window of about one square metre is raised. The window is situated at the south-west corner of the room.

The snails used were collected from the sandy shore, just before the experiment.

(1) *Movement in the dark room.* At first I wished to determine how *Batillaria* creep in the dark room. A glass plate was placed under a electric light, which is suspended from the ceiling. As soon as the snail was placed on the glass plate the electric light was turned off and 30 minutes later the traces were examined. I have tested 27 individuals using one in each test. Fig. 7 will show some of the tracings.

As is shown in Fig. 7, *Batillaria* shows a circular movement at the beginning of creeping. At the beginning some snails show right handed and others show left handed circular movements; 14 individuals out of 27 showed right handed and the remaining 13 individuals showed left handed circular movements. This initial circular movement is followed by either a spiral movement, or by a wave-like movement. 13 individuals showed a wave-like movement and 14 individuals showed a spiral movement. All the individuals which showed a spiral movement exhibited a right handed spiral movement. It is interesting to know that the shell of *Batillaria* is a right handed one.

The young individuals of about 8 to 9.5 milimetres in shell length, show nearly the same behaviour as the old ones. The diametre of the circle traced by the spiral movement of older snails were about 7 to 25



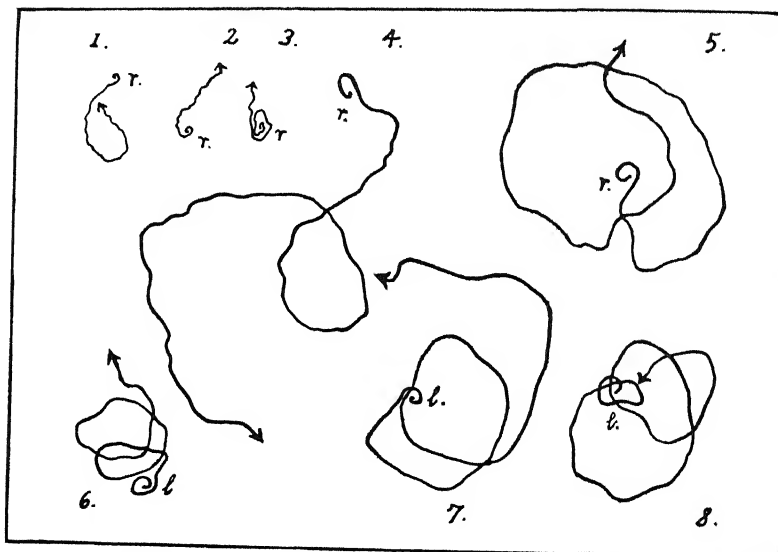


Fig. 7. Copies (1/8) of the tracks made by snails (*Batillaria multiformis*) in the dark.

1, 2, 3. tracks made by young individuals.

4—8. tracks made by older individuals.

l. left handed circus movement. r. right handed circus movement.

centimetres, while those traced by the younger snails were only 0.13 to 0.3 centimetres.

(2) *Phototropism in the room.* One *Batillaria* was placed on a glass plate, making an angle of 45 degrees with the direction of light, and one hour later the trace was examined. The results are given in Table II.

TABLE II.  
*Phototropism of Batillaria*

Animal number	Date June	Atmospheric temperature in C°	Angle of creeping in degrees	Angle of deflection to light in degrees	Phototropism
1	13	25.0	30.0	- 15.0	+
2	"	"	17.5	- 27.5	+
3	"	"	24.0	- 21.0	+
4	"	"	40.0	- 5.0	+
5	"	"	55.5	+ 10.5	+
6	"	"	160.0	-115.0	-
7	"	"	?	?	?

Animal number	Date June	Atmospheric temperature in C°	Angle of creeping in degrees	Angle of deflection to light in degrees	Phototropism
8	13	25.0	38.0	- 7.0	+
9	14	"	51.5	+ 6.5	+
10	"	"	36.5	- 8.5	+
11	"	"	57.0	+ 12.0	+
12	"	"	42.0	- 3.0	+
13	15	24.0	59.0	+ 14.0	+
14	"	"	58.5	+ 13.5	+
15	"	"	53.0	+ 8.0	+
16	"	"	43.0	- 2.0	+
17	"	"	58.5	+ 13.5	+
18	"	"	63.0	+ 18.0	+
19	"	"	47.0	+ 2.0	+
20	"	"	54.5	+ 9.5	+
21	"	"	41.5	- 3.5	+
22	"	"	41.5	- 3.5	+
23	"	24.5	26.5	- 18.5	+
24	"	"	33.0	- 12.0	+
25	"	"	49.5	+ 4.5	+
26	"	"	51.5	+ 6.5	+
27	"	"	34.0	- 11.0	+
28	16	"	40.0	- 5.0	+
29	"	"	63.0	+ 18.0	+
30	"	"	58.0	+ 13.0	+
31	"	"	54.0	+ 9.0	+
32	"	"	63.0	+ 18.0	+
33	"	"	45.5	+ 0.5	+
34	"	"	64.0	+ 19.0	+
35	"	"	19.0	- 26.0	+
36	"	"	46.0	+ 1.0	+
37	"	"	244.0	+199.0	-

No. of cases which showed positive phototropism

94%

In the column of "Angle of deflection to light", + indicates that the snail crept to left ward from the direction of light, and - indicates that of to right ward.

As are shown in Table II, 37 individuals were examined, and 34 showed positive phototropism within deviation of 2.9 degrees of creeping angle to light (between 27.5° to right and 20.0° to left). At the beginning

of creeping 15 individuals out of 35 showed a circular movement.

It thus becomes clear from the present test that *Batillaria* shows reversed reaction to light in the room when compared with the reaction shown in the field. There were several cases of exceptions in which the individuals numbered 8, 9 and 12 showed at first positive phototropism as other snails and crept about 30 to 40 centimetres towards the light but then turned their direction gradually away from the light and showed negative phototropism.

So it may be said that *Batillaria* shows random movement in the dark, because of absence of light to indicate the direction of creeping, and in the defused day light it shows positive phototropism, because of necessity of a brighter light<sup>1</sup>, and under the direct sun light, it shows negative phototropism, because of too strong a light. In this connection it will be interesting to study further the limit of the tropism which has not yet been tested.

*Batillaria* shows a circular movement in the dark without exceptions, but in the defused day light, it appears in 50% of them. So, I think that the circular movement appears to be due to the search for more comfortable shelter.

##### 5. Movement of *Batillaria* affected by stream (Experiment on rheotropism)

During low-tide, a shallow stream is formed on one part of the sandy shore near the Station. On July 2nd, at 3:00 pm, 86 individuals were found creeping the stream. In another case, 46 individuals per one square metre were creeping in the stream and in this case all of them were found creeping towards the stream. In the cases above stated sun light was directed towards the upper part of the stream. At this time, there were no snails creeping on the sandy shore, but tracings of recent burials by 3 to 5 individuals per one square metre were noted.

On the ebb tide, those *Batillaria* which remained within the reaching distance of in and out waves crept seaward suggesting the positive rheotropic reaction but when the tide recedes far away, they shows negative phototropism (the direction of sun light from sea to the land at this locality) and return shore-wards as above stated. The facts given above

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<sup>1</sup> According to the experiment carried out by Mr. SENJI SUZUKI of the Mitui Institute of Marine Biology, *Nerita japonica* DUNDER and *Monodonta neritoides* (PHILLIPPI) show negative phototropism under the same conditions.

suggest that *Batillaria* may possess a positive rheotropic nature, and the following experiments were carried on to test this idea.

A glass plate is inclined five degrees to the horizontal plane, and sea-water runs from the higher part of the plate in the rate of 350 to 400 cc per minute, and 5 individuals are placed on the lower side of the plate, each being separated 4 centimetres apart and their heads were directed towards the source of the stream. 30 minutes later their traces were examined. The results are shown in Table III.

TABLE III.  
*Rheotropism of Batillaria*

Animal number	Date	Atmospheric temperature in C°	Angle of deflection to the source in degrees		Rheotropism
			1st 10 cms	2nd 10 cms	
1	June 29	23.5	— 47.0	+ 8.5	+
2	" "	"	— 42.5	+ 19.5	+
3	" "	"	+ 6.0	+ 5.5	+
4	" "	"	— 18.5	+ 46.5	+
5	" "	"	+ 67.5	— 4.0	+
6	" "	"	— 35.0	— 1.5	+
7	" "	"	— 10.5	— 34.0	+
8	" "	"	+ 35.5	+ 119.0	+
9	" "	"	— 13.0	+ 38.5	+
10	" "	"	—	+ 132.0	—
11	" 30	27.8	— 2.5	+ 160.0	+
12	" "	"	+ 49.0	+ 130.0	—
13	" "	"	+ 35.0	— 12.0	+
14	" "	"	— 106.0	..	—
15	" "	"	— 47.0	— 27.0	+
16	" "	"	— 114.0	— 95.0	—
17	" "	"	+ 140.5	+ 92.0	—
18	" "	"	+ 81.0	— 90.0	—
19	" "	"	— 116.0	..	—
20	" "	"	..	..	..
21	July 2	29.4	— 8.0	— 1.0	+
22	" "	"	*+ 126.0	+ 107.5	—
23	" "	"	0.0	+ 3.0	+
24	" "	"	*— 20.0	— 60.0	+
25	" "	"	+ 12.0	— 174.5	+
26	" "	28.8	— 11.0	— 106.5	+

Animal number	Date	Atmospheric temperature in C°	Angle of deflection of the source in degrees		Pheotropism
			1st 10 cms	2nd 10 cms	
27	July 2	28.8	+ 10.0	+ 1.0	+
28	" "	"	- 69.5	+ 10.0	+
29	" "	"	- 9.5	- 29.0	+
30	" "	"	- 44.5	- 27.0	+
31	" "	29.0	- 3.0	+ 17.0	+
32	" "	"	+ 82.0	..	-
33	" "	"	- 1.0	+110.0	+
34	" "	"	- 22.0	+103.5	+
35	" "	"	- 26.0	+ 2.0	+
36	" "	29.6	- 82.0	- 82.0	-
37	" "	"	- 23.0	+ 5.0	+
38	" "	"	+ 15.0	+120.0	+
39	" "	"	+111.0	- 62.0	+
40	" "	29.9	-141.0	- 47.0	+
41	" "	"	- 25.0	+ 13.0	+
42	" "	"	+ 66.0	+ 66.0	+
43	" "	"	- 1.0	+ 21.0	+
44	" "	"	- 76.0	+ 23.0	+
45	" "	"	- 3.0	+ 60.0	+
No. of cases which showed positive rheotropism					77.7%

\* + indicate that the snail crept left ward, and - indicate that of right ward.

In Table III, we see that 35 among 45 individuals showed positive rheotropism and 10 individuals showed negative rheotropism. Differing from the values obtained in nature where 97-100% showed positive rheotropism while in the present experiment the positive reaction was shown by 78%. But I think the difference of about 20% just indicated may be accounted for an inadequacy of the method of the experiment. (In this experiment, I have noticed that at the beginning of creeping 38 individuals among 45 used a circular movement; right handed ones 22 and left handed ones 16.) At any rate, it may be said that *Batillaria* shows positive rheotropism both in nature and in the laboratory.

#### GENERAL CONSIDERATION

It is generally stated that the movement of many animals which inhabit the littoral zone is influenced by tide. K. MITSUKURI (1901) who studied

the nature of *Littorina exigua* stated that the locomotion of *Littorina* is due to its negative phototaxis and negative hydrotaxis. J. D. HASEMAN (1911) also studied on the rhythmical movement of *Littorina litorea* and says, "The primary directive force rhythmical movements is the surface film of water. The secondary directive forces are the quiescent position of desiccated individuals, character of the source, moisture and food." The present author (ABE, 1931) found with *Acmaea dorsuosa* GOULD that the boundary line between water and air strongly affect their movements.

The movement of *Batillaria* also seems to be determined by tide and the direction of locomotion is determined by sun light as already stated. So it follows that if the movement of *Batillaria* is determined solely by the two factors, sun light and tide, then it follows that the snails would perform a large semi-circular movement during 15 days by negative phototropism, and thus gradually migrate from the south part of the sandy shore to the north part. But in reality, the movement is influenced also by food, unevenness of the shore and shadow of the rocks or weather, consequently the theoretical semi-circular movement is much disturbed. Furthermore a shallow stream appears in the north part of the sand at the time of low-tide, and large number of them, by positive rheotropism, migrate to the east part of the shore (higher part). Therefore the combinations of all these varied factors above stated tend to scatter *Batillaria* here and there on the sandy shore.

Next, on the fact that the migration of the snails is most numerous on the days of half-moon than on the days of full-moon and new-moon, the reason is not yet clear.

In short, it can be said that the movement of *Batillaria* is influenced primary by tide, as HASEMAN says on *Littorina litorea*, and secondary influenced by negative phototropism under the direct sun light, as MITSUKURI says on *Littorina exigua*.

#### SUMMARY

1. *Batillaria multiformis* (LISCHKE) inhabits the littoral zone, and individuals are most numerous in the rock habitat than in the pebble and sand shore habitats.

2. Method of locomotion of *Batillaria multiformis* belongs to monotaxic direct movement, and moves by slow gliding mixed with leaping in regular intervals.

3. *Batillaria multiformis* begins creeping soon after the tide retires,

and 20 or 30 minutes later, on the sand it begins to prepare for burying its body in the sand and creeps in from the head side.

4. *Batillaria multiformis* do not move at the time of high-tide, and are found under the sand, the depth of the sand is less than three centimetres, the majority being in 1 to 1.5 centimetres in depth.

5. When low-tide occurs twice in a day time, once in early morning and once in evening, a larger number of *Batillaria multiformis* are found in the afternoon low-tide than in the morning low-tide.

6. Direction of creeping of *Batillaria multiformis* on the sand is determined by the sun light in the field by the nature of negative phototropism, but it shows positive phototropism in the defused day light in the room.

7. There are rhythmical changes in their movements and migration, and on the days of half-moon, the number of individuals are most numerous, and on the days of full-moon and new-moon, the number of individuals are least.

8. *Batillaria multiformis* shows random movement in the dark, and shows a circular movement at the beginning of creeping. This circular movement appears to be due to the search for a more comfortable shelter.

9. *Batillaria multiformis* shows positive rheotropism, and the great number of individuals are seen migrating in an upward direction of the shallow stream at the time of low-tide by this nature.

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